COURTSHIP AND HYBRIDIZATION IN THE *Drosophila nasuta* SUBGROUP

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ABSTRACT

Mayr's (1963) Biological Species Concept and Dobzhansky's (1951) 'reproductive isolating mechanisms' are central in contemporary theories concerning species and speciation. A species is viewed as an adaptive device, having diverged from related species in order to prevent loss (through hybridization) of adaptive advantage. Paterson (1985) rejects the idea that *ad hoc* isolating mechanisms can evolve and that species are a *direct* product of selection. Instead he focuses on *ad hoc* reproductive mechanisms and species as incidental consequences of adaptive evolution. With respect to the deleterious effects of hybridization, Paterson predicts that natural selection acts by eliminating the cause of negative heterosis; this represents neutralization, not improved isolation. Examples of this phenomenon exist but the present study is the first to focus on the fate of deleterious behavioural elements in hybrid swarms.

The male courtship behaviour of *Drosophila nasuta* and *D. kepulauana* differ physically, acoustically and in the characteristics of daily rhythm. However, when confined together, the two species will hybridize. Cross mating is biased towards the *nasuta* (female) x *kepulauana* cross. First generation hybrid males have uniform and intermediate courtship behaviour which, although vigorous, is relatively inefficient with both sibling and parental females. Hybrids were fertile and were confined together to form 49 populations. Individual F_2 males varied: starting position, interburst-interval, courtship song and the angle of wing-extension were elements which segregated independently. After many generations (10-45) the efficiency of hybrid courtship in each population was tested with *nasuta*, *kepulauana* and sibling females. Natural selection and drift had resulted in the restoration of the *nasuta* specific-mate recognition system (SMRS) in 67% of the populations, the *kepulauana* SMRS in 4% and mating systems inefficient with both *nasuta* and *kepulauana* in the remainder. Tests of sexual coadaptation in the latter

were inconclusive but in the former populations, mating occurred with non-hybrid efficiency.

The results show that populations become behaviourally homogeneous through time; parental mating systems were completely recovered. It is concluded that behavioural elements of one of the parent species are eliminated because in context with the mating system of the other they are deleterious. Behavioural context is the product of stochastic processes generating different fields of possibility in early-generation hybrid populations. Ad hoc reproductive mechanisms evolve or are recovered after introgression disrupts pre-existing mating systems; this evidence supports Paterson's Recognition Concept of species.

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

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Shane F. McEVEY (candidate)

..... day of ..... , 1988.

LIST OF ABBREVIATIONS

ibi	interburst-interval (section 4.8)
nas	Drosophila nasuta
kep	Drosophila kepulauana
hyb	hybrid Drosophila nasuta/kepulauana (see below)
AX	female acceptance posture
C	wing-extension, typical of active Courtship
FS	Female Stationary
FT	FasT or FlighT evasion, a female behaviour
OB	Orientation Begins between male and female
OE	Orientation Ends between male and female
OEX	Orientation interrupted by EXtraneous factor
TRACE	Tracking by Replay And Computer Eduction method
WA	slow, WAlking away 'evasion', a female behaviour
X	copulation
Ta	Time available, the time a fly is unencumbered
Td	duration of copulation, Td = Tec - Tsc
Te	Time at the end of a trial
Tec	Time of the end of a copulation
Ti	Time of insertion of a male into the arena
Tl	The time lapsed since male introduction Tl = Tsc - Ti
Tq	sex quotient, Tq = Tsex / Ta
Tsc	Time of the start of copulation
Tsex	Time spent courting, Tsex = ibi x (number of C's)

N.B. Throughout, the convention of writing female first is followed e.g., Drosophila nasuta/kepulauana is a hybrid from the cross between a nasuta female and a kepulauana male.

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PREFACE

'Bug hunting was the Trojan horse of Victorian agnosticism'
(Burrow 1968:19).

How to treat species depends 'upon whether one looks at them from the viewpoint of the taxonomist or the student of evolution' (Mayr and McEvey 1960:188). As a student of evolution and a taxonomist I rest uneasily with this dualism. My boyhood interest in bug hunting (guided with wisdom by my father) developed latterly into a more formal interest in the taxonomy of Drosophilidae. But that represented the beginning of a new interest and a new direction. The move I made from Ian Bock's taxonomy lab to Hugh Paterson's evolutionary lab represented for me a change in viewpoint but not a change in subject. Through the taxonomy of any group, especially one with Drosophila within its ranks, one arrives inevitably at evolution and Big Questions. From both Bock and Paterson, however, I learnt a very sound philosophy: not to search for direction in nature's course - she is godless. In addition I learnt that the whole study of life and its diversity is intricately interwoven with different philosophies. Consequently it is almost impossible to proceed without familiarizing one's self with history and methodological bias. Philosophy is not of facts and figures ... 'A quantitative, rather than a verbal, intellect would have written a very different book' (Ghiselin 1974:xi).

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1. INTRODUCTION

1.1 Introduction

In a long series of papers (1973-86), Paterson and his associates have examined critically the current Biological Species Concept of Dobzhansky and Mayr and have concluded that, as formulated, it is inconsistent and can be falsified on a major point (Paterson and Macnamara 1984). An alternative concept has been offered in its place (Paterson and James 1973; Paterson 1978, 1980, 1981, 1985, 1986). Paterson's Recognition Concept seems to be free of inconsistencies and evidently encompasses most biparental organisms. It is based on an almost axiomatic point, that all biparental organisms possess special adaptations to achieve fertilization. The essence of the new concept is that these adaptations are all that need be specified to delimit a species' gene pool. The notion in undeveloped form is old, but this is the first time that it has formed the core of a species concept which has been worked out in considerable detail. The fundamental idea is of course familiar to all biologists, but many, including Mayr, have failed to appreciate that it is quite different from defining species in terms of reproductive isolation from other species (i.e. relationally).

In motile biparental organisms, such as the species of Drosophila, positive assortative mating occurs as a consequence of the reproductive behaviour which Paterson (1978) has named the Specific-mate Recognition System (SMRS). The SMRS is part of the broader group of adaptive characters, the fertilization system, and comprises those characters involving signalling between mating partners or between their cells. Paterson (1978) predicted that the SMRS should be stable since it involves coadapted characters in the mating partners, and because the characters of the SMRS are appropriate to the normal habitat of the organism. This expectation is supported by the observations of Drosophila species by Henderson and Lambert (1982). Paterson (1982a, 1985, 1986) has shown that the stability of the SMRS effectively accounts for the

equilibrium phase (especially in sexual characters) of the punctuated equilibrium pattern in palaeontology (Eldredge and Gould 1972).

The Recognition Concept yields predictions relating to the fate of hybrids (Paterson 1978, 1986; Harper and Lambert 1983), and it is the in depth empirical examination of these predictions that forms the basis of the present study.

1.2 Statement of the Problem

For many the most familiar species definition is Mayr's: ' ... groups of interbreeding natural populations that are reproductively isolated from other such groups'. That a single view of species holds sway is evident in numerous biological texts which adopt Mayr's definition as if it were irrefutable.

Paterson (1982b) showed that the idea of defining species in terms of their ability to avoid hybridization was an old one tracing back at least to Lyell (c. 1832) via an intellectual pedigree including Wallace, Gulick, Robson and Fisher. In fact the significance of hybridization can be traced even back to ancient animal and plant breeders in Africa (see Pernes 1983 for example). Paré's Monstres et Marveilles, a bizarre semi-scientific treatise of teratologies first published in 1573, documents numerous examples of half human half beast creatures, each the imagined product of bestial (hybrid) sexual relations and each an example of the wrath of God (Pallister 1982). Even today the view that hybrids are 'bad' exists as a cryptic bias which pervades the understanding of species and speciation. It is deep-seated and difficult to be rid of and is behind a number of ideas in the study of evolution (Ghiselin 1974, Monod 1970).

To suggest that mechanisms evolve to ensure reproductive isolation is a line of reasoning which has been expressed in genetic terms this century, giving rise to the neo-Darwinian paradigm. Dobzhansky (1951), the leading protagonist, defined species as 'groups of populations the gene exchange between which is limited or prevented in nature by one or a

combination of several, reproductive isolating mechanisms.' Dobzhansky's satisfaction with this interpretation may arise from his conviction that 'The hypothesis of mechanism has triumphed everywhere ...' (Dobzhansky 1967:19). He seems to equate rejection of vitalism (1967:20) with emphasis on mechanisms. But his error, in my opinion, is that speciation is more likely a fortuitous or accidental process requiring no mechanism. If natural selection is creative by accident we are not compelled to find either a mechanism or a vital force behind the diversity of life.

Note how there is no fundamental novelty about a notion proposing that mechanisms evolve a priori by natural selection to protect the integrity of a species gene pool or one like Ray's (1727) proposing that 'the great design of Providence (is) to maintain and continue every species', or Lyell's notion that natural 'causes' exist intervening to prevent 'conversion' of species, (Lyell 1835) i.e. 'sterility protects God's handiwork'. Dobzhansky (1967) attempts to draw a sharp division between his own philosophy and vitalism and yet the above similarity remains. Dobzhansky has adopted a kind of animist projection, Monod (1970:46) writes: '(the) attempt to systematize a subjective interpretation of nature, showing it to have an ascending, constructive, creative intent, a purpose; in short, to make nature decipherable and morally meaningful ... is "animist projection" ... always recognizable whatever its disguises.' This corresponds to Paterson's (1982b) observation 'That Darwin's great achievement of freeing biology of teleology and the supernatural, (has) been surreptitiously supplanted by the old theological concept deceptively clad in new clothes'.

Dobzhansky (1976), in fact, suggested that species are not accidents but 'adaptive devices' having evolved mechanisms serving the special purpose of preventing the 'submergence of existing genetic systems' (Dobzhansky 1937). The implications that arise from this are certainly important if one wishes to understand species and speciation without ambivalence. The extent of such a philosophy in contemporary biological literature is astonishing once noticed.

Establishing that contemporary evolutionary theory has dubious underpinning is one problem. Another is the derivation of predictions which are open to refutation. The current paradigm appears to be teleological and not open to logical investigation. Furthermore, given the historical prejudices, it is likely that, even if refuted, acknowledgment of that fact will come only reluctantly (Kuhn 1970).

How then, is the best way to begin? Paterson (loc.cit.) has suggested a new definition which is more in keeping with Darwin's original view of an accidental, non teleological, origin of species. The new definition denies that species have a purpose and sex is given a key role. Indeed Paterson has found that the adaptive system which leads to syngamy is sufficient in itself to define a species. The definition does not concern itself with the relatedness of one species to another or the ability or inability for individuals to rate potential mates as superior or inferior; these properties may be of incidental importance. The achievement of syngamy, however, is of primary importance, it is the raison d'être of sexual organisms and is an end point of a tightly coadapted chain of signals and responses that impinge on an organism during the span of its reproductive life. It is not something that 'implies consciousness' on the part of the interacting animals or plants, nor is it a quibble over 'terminology' (Mayr 1985:308).

Obviously it is true that in nature syngamy occurs between particular pairs of gametes and that natural selection will tend to favour the communication system that works 'best' in the organisms' normal habitat. Paterson's concept of species is summed up as: 'that most inclusive population of biparental organisms which share a common fertilization system' (Paterson 1985, 1986).

Several evolutionists are beginning to see merit in Paterson's new concept (Templeton 1987 cf. 1980, Carson 1987). But there are many from the Isolationist school who reject or fail to understand the underlying shift in theory (e.g., Butlin 1987, Templeton 1980, Littlejohn 1981, Mayr 1985). For general understanding it is regrettable that Paterson's ideas

are occasionally and carelessly incorporated (Butlin 1987) into the very paradigm he so rigorously seeks to undermine.

New ways of thinking invariably meet with this sort of resistance, misunderstanding and forced conformation and it is typical for protagonists of an old paradigm to attempt to fit ideas from the new into the old framework (Kuhn 1970). There are also those who, after having studied the arguments (e.g., Littlejohn 1981, Mayr 1985), conclude that semantics alone distinguish them and that no radical difference actually exists. In answer to this common criticism, Paterson precisely stated (1982a) the incongruity of his and the contemporary concept of species: In essence the Mayr-Dobzhansky school either directly or indirectly imply that one species does not interbreed with another because mechanisms have evolved to serve the **function** of reducing hybridization and maintaining the species' genetic integrity. While Paterson argues that limited hybridization is an **effect** of the evolution of mechanisms evolved to achieve syngamy in a particular habitat.

It is apparent that Mayr had difficulty with ad hoc natural mechanisms, he could not satisfactorily explain how allopatric speciation could account for their evolution. Paterson suggests that it is highly unlikely that mechanisms evolve to serve the function of reproductive isolation (see Spencer et al. 1986). If signals and responses of two organisms fail to result in syngamy, this he interprets as an **effect**. The Mayr approach with emphasis on reproductive isolating mechanisms leads to a **relational** concept of species, the Paterson approach, with an emphasis on fertilization (and low heterogamy as an effect) to a **non-relational** concept; philosophically there can be no doubt that they are radically different.

Mayr and Dobzhansky have concordant ideology but Mayr rests uneasily on his theoretical foundation. Of divergent behaviours and morphologies Mayr (1963:548) wrote: 'They are ad hoc mechanisms. It is therefore somewhat difficult to comprehend how isolating mechanisms can evolve in isolated populations.' The awkward problem Mayr faces here is simply that of how mechanisms, designed to perform a special

function, evolve when the function to be performed lies in the future? But at the same time he states, and therein reveals his inconsistency, that reproductive isolation is brought about by '*ad hoc* mechanisms' i.e. mechanisms with a special purpose. If reproductive isolating mechanisms evolve for the special purpose of maintaining genetic integrity then species never evolve in allopatry but always in sympatry. This is clearly not the case and thus the major flaw is revealed. Therefore Mayr's definition (above) is hypothetically relevant only for species which evolve in sympatry and yet Mayr, and biologists generally, maintain that the allopatric mode of speciation is the most likely (Paterson loc.cit., Dobzhansky 1970, White 1978, Mayr 1963, Bush 1975, Grant 1971).

To test the Mayr-Dobzhansky theory, experiments were conducted to determine whether strong artificial selection could reinforce a weak initial tendency for a species-pair to mate assortatively. The most often cited of these experiments are Koopman's (1950) involving the closely related species *D. pseudoobscura* and *D. persimilis*. Of Koopman's work Dobzhansky (1951:209) writes 'a striking experimental verification ... Promiscuity was, consequently, made selectively disadvantageous, and mating within the species adaptively advantageous'. A large number of similar experiments have been undertaken (see Petit 1980 for review). The success of some of these was - and is still - cited as experimental 'proof' that species can evolve in relation to other species. But serious flaws in design (Paterson 1978) and inconsistencies of logic (Paterson 1978, Spencer *et al*. 1986) have been detailed. If positive assortative mating were ever shown to occur throughout a natural population as a direct consequence of natural selection eliminating outbreeders it would be called Speciation by Reinforcement. However, it has not been unequivocally demonstrated although a great number of examples of reproductive character displacement in parapatric zones are cited. The problem with the latter is that behavioural or morphological alteration at a hybrid zone has not been shown to spread to individuals in allopatry, this is an important requirement (Moore 1957). In any case most of these examples

of character displacement or equivocal (Grant 1972) and Speciation by Reinforcement remains just a 'fascinating theory' (Carson 1987:597), interesting but untrue.

The Recognition Concept emphasizes that mechanisms for achieving fertilization have a stabilizing effect on a population, this is given as the reason why species are static after speciation and it correlates with historical phylogenetic patterns of equilibria punctuated by speciation events. Moreover it is predicted that a population (e.g., a hybrid swarm) in which individuals are not co-adapted between the sexes is unstable or metastable (sensu Li 1955). During such instability certain elements (e.g., genes, chromosomes, signals or responses) are in a state of flux and it is predicted that they are eliminated or retained from the population depending on whether they effect fitness. If a male and female possess elements which are deleterious only when combined in their offspring, no mechanism exists to prevent their union; the reduced fitness of the heterotypic progeny has the effect of removing from the population the element that caused the negative heterosis. Deleterious elements are eliminated from the gene pool and mechanisms for achieving reproduction prevail or the population drifts to extinction. In extant introgressed populations the negative effects of heterosis are thus neutralized rather than prevented. Elements which have limited effect on fitness may persist. In an old hybrid swarm individuals may possess elements from both founder populations (or parents). The present study is an investigation of these predictions in artificial hybrid swarms of the behaviourally different species D. nasuta and D. kepulauana.

1.3 Method of Investigation

What happens when potentially interbreeding individuals of two incipient species meet and hybridize? How does natural selection 'create' from the disassembled parts, what is the outcome in the game or '*jeu des possibles*' (Jacob 1981)? In the experiments which form the basis of the present study, two closely related species of *Drosophila* were forced to hybridize; their fertile progeny were grouped together in a number of separate bottle-populations and monitored for many generations. *Drosophila* are useful research organisms in this respect because laboratory confinement does not adversely effect their sexual behaviour. In the final analysis, after many generations, the efficiency of their sexual behaviour is compared to that of their non-hybrid parents and in this way it is possible to gain some insight into the likely outcome of hybridization between sexually divergent species in nature.

The difference between the present study and earlier ones of the Koopman or Wallace type (see above), is that sexual characters rather than just morphological characters are scrambled, populations comprise only hybrids and here there is no artificial selection of types nor manipulation of ratios. During the course of this study a work was published by Wallace *et al.* (1983) which appears to have a similar objective. However, rather than examining the outcomes in a large number of populations, Wallace and his colleagues tested only four; they worked with *D. pseudoobscura* and *D. persimilis* which have no visibly divergent courtship behaviour (cf. *D. nasuta* and *D. kepulauana*) although they mate assortatively. In contrast 50 populations were assayed in the present work. In this way it was possible to observe a range of possible outcomes.

The courtship (*sensu* Morris 1956:261) behaviour of flies in the *D. nasuta* subgroup are elaborate displays which have been described by Spieth (1969). Additional observations are presented in this study. The courtship of *D. nasuta* was studied under natural conditions in Swaziland and two other related species (*D. sulfuriqaster* and *D. niveifrons*) were

studied in northern Australia (results not presented). It was possible to confirm, qualitatively at least, that behaviours in the laboratory also occur in the wild.

Before major behavioural assays it was necessary to determine the basic reproductive biology of nasuta and kepulauana: the rate of maturation and the timing of the egg-adult-egg cycle, the temporal sexual activity pattern and the age of sexual maturation in males and females. Apart from Spieth's description of courtship patterns the behaviour of these species have not been extensively studied although much work has been done on their chromosomes (Wilson et al. 1969, Wakahama and Kitagawa 1972, Lambert 1976, Singh and Gupta 1979, Takanashi 1983, Kitagawa et al. 1978). Wright (1977a,b) and Balwin (1983a,b) give some very cursory notes about diurnal variation in courtship activity and sexual receptivity. The discovery, in the final year of the study, of hybrid dysgenesis between subspecies of D. sulfurigaster led to the isolation of several eye-colour mutants. With such genetic markers, experiments on sperm utilization were possible and results are presented in Chapter 4. Additional work on the associated dysgenic phenomena is not presented in entirety; some data about the exceptional pattern of inheritance and sexual mosaicism are given in Chapter 3. The likely origin and pattern of subsequent dispersal of both the nasuta subgroup as a whole and certain species in particular, have an important bearing on the discussion of the asymmetric mating behaviour of nasuta and kepulauana, their possible allopatric isolation, and their speciation. For this reason a review of the biogeography of the species of the subgroup is presented.

Drosophila kepulauana and D. nasuta interbreed asymmetrically; this has complicated the interpretation of the mating success of advanced hybrids. Their efficiency must be compared with non-hybrids and the outcome scored in such a way that it indicates whether the hybrids are like one or other of their parent-species or whether they are different. The term 'isolation index' is inadequate in this context. Wallace et al. (1983) discuss the problem of the measurement of mating

success of one type among three. They introduce 'preference index'; but the word 'prefer' implies an ability to perceive two or more things and then to choose one whereas it seems less controversial to avoid altogether the use of 'choice'. The idea of courtship efficiency is introduced in Chapter 6. An index rates the males of a population on a scale of 0 to 1 so that comparisons can be made with nasuta males (rated 0.00) and kepulauana males (rated 1.00).

An important part of this thesis is the introduction of a new technique, called TRACE, for assessing courtship efficiency. The method yields information about the interactive activities of individual flies in unmarked groups. TRACE was developed principally to examine the nature of the inefficiency of first generation hybrid courtship but it was found to be a useful procedure in other analyses. No computer software was available for handling large data sets so a standard 'no-choice' method was used for testing the many advanced hybrid flies.

A taxonomic review of the species chosen for this study was necessary because their similar morphology and their ability to interbreed in captivity have led to a great deal of confusion. Many synonyms have been described in the subgroup, even since the major taxonomic review of Wilson et al. (1969). Many workers treat D. albomicans Duda, 1923 as a third species in a so-called nasuta - kepulauana complex, but in section 2.4 it is argued that albomicans is a synonym of nasuta. The scope of the problem of species concepts in taxonomy and in evolutionary biology is addressed below and it will be made clear why the species problem is fundamental in biology - it is related to the way we view life.

2. SPECIES CONCEPTS AND BIOGEOGRAPHY

2.1 Introduction.

Any scientific discussion should at the very least attempt to avoid ambivalence. Ambivalence frequently occurs in zoology when taxonomy is not clearly distinguished from evolutionary and population genetical studies. The present study is a population genetic study and is more concerned with evolution than with taxonomy. But, of course, the names which will be used for the species in this study derive from taxonomy and have implications which are sometimes incompatible with our knowledge of evolution. In many of the articles about the flies in the Drosophila nasuta species subgroup there is conflation of different meanings and uses of the word 'species'. In order to avoid such confusion a distinction will be made firstly between taxonomic and genetic species concepts in general and secondly between two particular genetic species concepts. One of the genetic species concepts (i.e. Mayr's Biological Species) will be shown to be falsifiable on a major point and the other (Paterson's Recognition Concept loc. cit.) will be shown to be logically consistent with current population genetics and natural selection theory and evidence.

It is possible to argue that two incompatible philosophies pervade present-day biology, one is Classical the other Darwinian or post-Darwinian. The Classic notion of the living world was based on ideas, immutable essences, and truth through definition. On the other hand the post-Darwinian understanding of organismic diversity derives from a knowledge of (1) the consequence of descent with modification and (2) similarity by virtue of relatedness. Problems occur when moving from taxonomy which is based on classes of individuals and immutability to population biology which is based on the flow of genes and changes to gene frequency. The science of systematics (sensu Simpson 1961) which has to a very large extent been promoted by Mayr, endeavours to link these disparate objectives and it may be argued that his Biological

Species Concept is a product of the same endeavour. It is the intention of the following discussion to justify the application of Paterson's Recognition Concept of species in this study and to place it in an appropriate philosophical context.

2.2 Taxonomic Species Concept

Following Malthus' Essay on Populations, Darwin and Wallace apparently gained a crucial insight while they pondered on the powerful effect of mortality on rapidly expanding populations. They were able to conceive of species in a manner totally different from the traditional view which had prevailed since Plato (Mayr 1963, Ghiselin 1969). What this revelation was has never been adequately explained but Ghiselin (1969) believes it may be summed up in a single word: **population.** This dynamic (cf. static) concept had a profound effect on the classification of the living world. So deep were the ramifications for classification that the Darwinian revolution effectively 'depended on the collapse of the Western intellectual tradition' (Ghiselin 1969). The concept of species changed in meaning during this period. This section is about the pre-Darwinian view of species as classes of similar things (taxonomic species), the concept which prevailed before Darwin and continues to manifest in present-day taxonomy and systematics. The ambivalence which arises as the result of this overlap is briefly discussed. In contrast the two following sections are about species as groups of genetically related entities (genetic species). The taxonomic species category has practical value in classification but it is not necessarily dependent on evolutionary thinking; the genetic species derives directly from evolutionary thinking but it does not readily provide information upon which classifications might be built, nor is it meant to.

Cladism is a phylogenetic taxonomic theory by which organisms can be ranked and classified exclusively according to 'recency of common descent' (Hennig 1966). It is therefore sometimes referred to as an 'evolutionary classification'.

There is a major fallacy however (Mayr 1969): relationships between organisms are not just products of branching, they are also products of subsequent divergence. Darwin (1859) makes this clear, he writes: 'I believe that the arrangement of the groups within each class ... must be strictly genealogical in order to be natural; but that the amount of difference in the several branches or groups ... may differ greatly, being due to the different degrees of modification which they have undergone ...'

The ideas which dominated theories of classification before the discovery of organic evolution have been called Essentialism by Popper. Essentialism follows Platonic logic. To Plato and Aristotle reality existed in the essence, Idea or eidos of a thing. A description was an Idea and was called a 'definition'. The truth could be reached through precise definitions. Therefore, more than just abstractions, definitions were a form of 'realism' (Ghiselin 1969). The search for distinguishing physical traits in taxonomy derives from essentialism and it is very important to realise that because essentialism is to do with classes it is completely inappropriate as a means for understanding individuals or populations (Hull 1976). Individuals and populations have similar properties (Hull 1976) they are 'born' or 'begin', they grow and change and they die or become extinct. But our concept of classes is rigid; once defined classes do not change - bottles are bottles even if they are all broken or change shape or are fashioned from plastic instead of glass; they can disappear and reappear (cf. individuals which exist only once and never reincarnate); a bottle nor a group of bottles have properties peculiar to individuals or populations (Hull 1976). In this sense a taxonomic species, as is sometimes understood by a binomial like Drosophila kepulauana, is like the variety of things called bottles. A taxonomic species is fixed: it cannot change or evolve; there is no such thing as taxonomic speciation! The species as understood in taxonomy should not be confused with the species as defined in terms of what a species actually is. One final point is that species when viewed not as a group but as a sort of larger-scale

individual avoids the need to discover a way to distinguish one species from another, individuals are autonomous and require no relative definition; they may be given a 'proper name' which is devoid of class connotation (Hull 1976). If species are individuals we need only discover the cohesive interactive mechanisms in order to know what a species is; a point to which I will return in section 2.3.2 on the Recognition Concept.

Nominalism stands in sharp contrast to essentialism. It 'asserts that classes are mere conveniences, artifacts of man's thought processes' (Ghiselin 1969:51); classes are not real, just useful abstractions. This is to some extent the contemporary view; nowadays 'definition' is considered to be of names or symbols (e.g., bottles) and the names or symbols designate things or essences i.e. the definition is not the essence. Thus we may 'define' Drosophila nasuta by giving it a precise description but by doing so we are not defining what a species is and through the definition we do not discover reality. We are merely catering to our desire to give it a tag. For example, we do not know Drosophila nasuta as a field for genetic recombination when we define it as having a jointed body, six legs, two wings, 2n = 8 chromosomes, frontal pollinosity, pleural darkening, sexual dimorphism, 'circular' courtship and an inability to breed with another Drosophila.

To a nominalist the process of classification involves no more than the arbitrary grouping together of sense data into classes which are treated as mere abstractions. Thus the classification of living things would be similar, in principle, to the classification of inanimate objects, like bottles. The phenetic approach of Sokal and Sneath (1963) is perhaps the clearest example of nominalism in modern biology; they would treat members of a family as if they were just as much abstractions as the class of things that have two legs, four legs or six or which fly in the air, move on the ground or swim in the seas. Avise (1976), Ayala (1975) and Lewontin (1974) are basically nominalist when they 'make taxa' on the basis of genetic distance or difference. For them two species exist when, for example, a set amount of genetic change has

occurred between them (but see Lambert and Paterson 1982). Lewontin (1974) has suggested that the genetic difference between sibling species will always be greater than 10% of the total genome as reflected by electrophoretic studies.

'The fatal weakness of nominalist thinking, when applied to the classification of organisms, is the reversal of an existing causal relation between "similarity and affinity"' (Mayr 1969:68-69). A species 'is not a strictly nominal class - that is, it is not an abstraction or mere group of similar things - because the biological individuals stand in relation to the species as parts to a whole' (Ghiselin 1969:53-54). Some species are real. While acknowledging these obvious shortcomings Ghiselin advocates that 'a certain dose of nominalism is ... most desirable in preventing the errors which result from thinking that a definition can be true or false' (1969:51). While Mayr (1969) seems to reject nominalism altogether. Certainly the existence of striking sexual dimorphism is an obvious problem in nominalist species classification.

There is, however, wide agreement that neither essentialism nor its logical opposite - nominalism - paved the way towards acceptance of natural selection and evolution. Essentialism was incompatible with evolution because essences, like classes, are immutable and cannot evolve. This fits the biblical story of Creation. 'If species change, they do not exist, for things that change cannot be defined (by the essentialist) and hence cannot exist' (Ghiselin 1969:53). Nominalism, on the other hand, is incompatible because evolution implies that certain assemblages in nature form real groups - natural groups or clades - and are therefore not mere abstractions.

The modern classification of the living world is based on that of Linnaeus (1707-1778). Linnaeus was an essentialist. For him classes and reality were one and the same. Differences between individual organisms were disregarded; members of a class were considered to be replicas of the Platonic eidos (this is typology which has been refuted by Simpson 1961 and Hull 1965). With this metaphysical preconception there is no

room for thinking about change which is reflected through the variability of individuals. On the other hand, for a radical nominalist, 'species cannot exist in principle, and any study of change must treat only individuals' (Ghiselin 1969:53). However, it is possible to embrace a kind of nominalism if one agrees with Hull (1976) that taxonomic species are a type of individual, they do not then require arbitrary class definition.

The result of Darwin's 'great conceptual leap' - his view of life as dynamic and transitory - has affected our understanding of the living world and the diversity of species. But for many there is no significant alteration of the old conventions of naming, defining, describing, or identifying species. The outcome is a new philosophy based on genealogy (i.e. relatedness), natural selection and change and the flow of genes in populations but it is superimposed on an old practice based on essential qualities, definitions, hierarchical classification schemes, the fixity of species and physical similarity. Darwin (1859) writes: 'Once objects were thought to be understood if they could be defined. Definitions were the reality and the entities were ephemeral. Subsequently it was realized that there was order in nature and that this order could be discovered ... in other words, instead of relying on similarity as a criterion in classification we now rely on relatedness.' The fundamental differentia changed from similarity to relatedness and the word species took on a new meaning. No longer were species classes of morphologically similar things they were now considered as groups of genealogically related entities. Thus instead of defining species we discover them.

Probably because there was often good correlation between the morphological species (species taxa or taxonomic species) and the species as a population of related individuals the Linnaean hierarchical system continues to be used despite its inappropriate basis.

Taxonomy is the science of classification and naming of organisms and has at its centre Linnaeus' hierarchical system of categories (i.e. species to kingdoms). It is a continuation

of pre-Darwinian methodology. Systematics, on the other hand, 'is the scientific study of the kinds and diversity of organisms (embodying the classic view of taxonomy) and of any and all relationships among them (recognition to Darwin).' (Simpson 1961, my brackets). It is therefore a science with disparate objectives; it attempts to define species as classes of physically similar entities, like bottles, and, at the same time, it tries to understand them in terms of their phylogeny and their genetic properties. Efforts to reconcile these discordant aims produce a 'warped and largely erroneous view' of the living world (Ghiselin 1969). My view is that systematics should be abandoned; no better understanding of the living world results by combining the three disciplines: taxonomy, evolutionary genetics, and phylogeny.

In taxonomy the word 'species' is a homonym and this is frequently overlooked. At once it is both a category (the 'species level') in the Linnaean hierarchy and the thing placed in that category. The thing in the category is a taxonomic class defined in a way that allows it to be distinguished from other taxonomic classes and this usually involves a physical identifying-character but may also involve something less tangible like a genetic or chromosomal trait which may also be useful in identification. Taxonomic species (i.e. species taxa) may sometimes coincide with a third 'kind' of species - the genetic species (e.g., Paterson 1985); it is the contention here that the genetic species is real whereas the other types are not, but they will sometimes coincide with the genetic species.

2.3 Genetical Species Concepts

Dobzhansky (1951:256) in his classic book: Genetics and the Origin of Species, treats the philosophical transition from taxonomic species to genetic species in the following way: 'Categories of classification are constructs devised by the student for his convenience; in this sense they are sometimes called "arbitrary" or "subjective". Mendelian popula-

tions are spatio-temporal objects and hence can be designated as "real" or "objective". But some taxonomic groupings ... are, by a deliberate effort of the systematists, made to coincide as closely as possible with Mendelian populations of different orders.' But, he emphasized, (1951:261) 'Mendelian populations are not synonymous with any groupings or categories of systematics.'

In this very influential work Dobzhansky forms our modern understanding of species as natural units which are genetically adherent or integral. He writes (1951:254) 'The potentially possible gene combinations constitute ... the "field" within which evolutionary changes may occur' and on p. 260 he reiterates an important and now very widely accepted notion that a species is '... the field of potentially possible gene combinations'. This term was first introduced by Sewell Wright (1932). Carson (1957) was later to use similar wording: 'a field of gene recombination' to express the nature of a species. Dobzhansky restricts genetic species definitions to sexual organisms and he emphasized the importance of bonds between individual organisms in a reproductive community (p. 260). These tenets are compatible with all modern concepts of genetic species. However, controversy arises when he, and many biologists in his footsteps, try to determine (1) the limits of this field and (2) how the limits originate ab initio (Paterson 1985, 1986). It is possible to distinguish Mayr's (1963) Biological Species Concept (= Isolation Concept) and Paterson's (1978, 1980, 1982a, 1985) Recognition Concept by the way the two resolve this problem. In the following discussion the objective is to demonstrate the inconsistencies involved in the former concept and the advantages of the latter.

2.3.1 Isolation Concept

Mayr (1976) credits Dobzhansky (1937) for having introduced 'isolating mechanisms' into the study of evolution. This term actually appears first in a relatively unknown 1935 pub-

lication (Dobzhansky 1935). Dobzhansky (1951:262) attributes the qualifying term 'reproductive' to Mayr (1942). As early as Gulick (1905) and Robson (1928) 'isolation' has been used in the context of species evolution. The term '**reproductive isolating mechanism**' has become so widely accepted in the study of evolution that it and the accompanying theory has acquired the status of a paradigm. Paterson (1980) calls the Mayr-Dobzhansky paradigm the Isolation Concept; under this view species are regarded as 'adaptive devices' (Dobzhansky 1951, 1976; Mayr 1949) whose integrity is maintained through the possession of reproductive isolating mechanisms. Species are defined either as 'groups of populations the gene exchange between which is limited or prevented in nature by ... reproductive isolating mechanisms' (Dobzhansky 1951:262-263; see also Dobzhansky 1970). Or they are defined as 'groups of actually (or potentially) interbreeding natural populations which are reproductively isolated from other such groups' (Mayr 1969:412, see also Mayr and Provine 1980). These definitions are essentially the same; in them, species are defined in **relative terms.** Note that the the word 'relative' has a different meaning here compared to when it was used above in the discussion of genealogical 'relatedness'. In the latter sense it is equivalent to 'allied by blood'; in the sense of 'reproductive isolation' it is equivalent to 'in relation to, or, with reference to something else'. In this second sense it has similarities to a taxonomic character, a feature which may be used by man for identification. It should be noted that Darwin rejected the sterility test and essentialism and therefore it may also be said that the Biological Species concept is not conceptually the same as the species as understood by Darwin (Ghiselin 1969). Both Mayr and Dobzhansky imply that reproductive isolating mechanisms set the limits of the field for gene recombination. Species are defined with reference to other species; their definition is therefore relative.

But how do 'isolating mechanisms' arise initially? If isolating mechanisms are indeed ad hoc characters, as stated by Mayr (1963:548), they are adaptations, as the appellation

'mechanism' implies (Paterson 1978, 1985; Williams 1966; Carson 1987). For such mechanisms to evolve, speciation must be a sympatric (or parapatric) event. However, it seems to be universally accepted (even by those who advocate sympatric speciation [Bush 1975, White 1978]) that speciation in total allopatry is the 'normal' mode. Other modes are purely hypothetical or based on scant evidence (Paterson 1978, Templeton 1987). Both Mayr (1963) and Dobzhansky (1951) explicitly deny the notion that species must always be 'intersterile' but having explicitly disclaimed something in one place and then to imply it in another, as they do, is quite simply ambiguous. Furthermore, the line of reasoning offered by Mayr and Dobzhansky has overtones of group selectionist thinking. The mechanisms are supposed to protect the genetic integrity of the species! This view of the living world has a very ancient pedigree; with an epistemological lineage connecting Mayr, but more particularly Dobzhansky, to Wallace, Lyell, Ray, the Bible, and even to ancient practices in animal and plant husbandry (Paterson 1982b, Pernes 1983:915).

Thus a paradox exists which neither Dobzhansky nor Mayr satisfactorily resolve. Reproductive isolating mechanisms 'are ad hoc mechanisms. It is therefore somewhat difficult to comprehend how [they] evolve in isolated populations' (Mayr 1963:548). The equivalent problem occurs to Dobzhansky (1970:376). Both leave the issue in a state of confusion and the problem persists. Perhaps, as Paterson (1985, 1986) believes, no satisfactory solution is possible. The logical inconsistencies which Mayr in various publications attempts to reconcile or overlooks altogether are summarised by Paterson (1986:63) in the following passage: 'It is curious that Mayr (1963:551) should write: "The thesis of the origin of reproductive isolation as a by-product of the total genetic reconstitution of the speciating population is consistent with all known facts." If this were so, how can he believe that speciation is an adaptive process (Mayr 1949), or that isolating mechanisms are ad hoc characters (Mayr 1963) which function to protect the genetic integrity of species, or that in the absence of close congeners isolating mechanisms can "afford"

to be "general, non-specific, and variable" (Mayr 1963:109). If Mayr really accepted the view that isolating mechanisms arose through pleiotropy, they are clearly mis-named and should be renamed "isolating effects" sensu Williams (1966).'

Thus if the limits of a field for gene recombination are set by isolating mechanisms it is not at all consistent to talk of allopatric speciation as the means by which such mechanisms arise. Problems of 'function, logic and consistency beset the Isolation Concept' (Paterson 1985). It is flawed on a major point: reproductive isolation is an effect, it cannot be a mechanism or adaptation.

2.3.2 Recognition Concept

When Dobzhansky (1937) introduced the Isolation Concept it was quickly accepted. If the concept had represented a new paradigm it should have been greeted with much greater resistance and confusion (Paterson 1985), more like the aversion and opposition which greeted Darwin's ideas (Kuhn 1970). Because it was immediately accepted, Paterson, in his search for a logically consistent species concept, was prompted to consider 'the subliminal preconceptions which predispose us to thinking of species in relational terms' (1986:63) since he had observed that Dobzhansky's was a relational definition (Paterson 1985). Some of these preconceptions clearly derive from essentialism and nominalism which have been described above. For example, Darwin's (1859) rejection of the sterility test is in many ways a result of his having abandoned essentialism (Ghiselin 1969:95). The covert resurrection of the sterility test this century, whether or not it comes in the guise of some other term (e.g., speciation by reinforcement, character displacement, mate choice) is effectively a return to pre-Darwinian ways of thinking about species.

The origin of sexually autonomous organisms must have been associated with special mechanisms for bringing about reunion and fertilization (Peterson 1905, Darlington 1964). Even for sexual organisms which are not completely independent

it is quite clear that syngamy is a process which will not occur sufficiently often by chance. One only has to consider the size of a gamete, the vastness of the biosphere and the probability of chance encounter.

If one accepts Carson's view of species as a field of gene recombination, then it is necessary to restrict discussion to those organisms which are, at least, facultatively biparental. This means that sex is fundamental to understanding species; it is the key to a genetical species concept. Every biparental organism possesses adaptations which comprise a system which leads to fertilization occurring (Paterson 1986). In motile sexual organisms 'there is a particularly important subcategory comprising signal-response characters in the mating partners: the specific-mate recognition system (SMRS)' (Paterson 1978, 1980, 1985). 'The raison d'être of the SMRS is to achieve effective fertilization under the conditions set by the organism's normal habitat, way-of-life ... and during its normal breeding season' (Paterson 1986:63). The SMRS is, of course, subjected to natural selection. The word 'recognition' means 'the response of one mating partner to a signal from the other' (Paterson 1982a). Recognition is thus the specific response by one partner to a specific signal from the other (Paterson 1985). Paterson uses the analogy of the recognition of a specific antigen by its specific antibody to emphasize that he implies no act of judgement or choice on the part of the responding partner (Paterson 1982a).

He suggests, as a working hypothesis, that all steps in a fertilization system have evolved to serve a particular purpose (Paterson 1985). In other words, that they are or were adaptations sensu Williams (1966).

With respect to the problem of how to delimit the field for gene recombination under natural conditions, it is clear that the characters of the fertilization systems are relevant. 'It is the fertilization system which leads to the positive assortative mating that delimits the field' (Paterson 1985:25). Reproductive isolation is seen simply as an effect. The fertilization system comprises components which are subject to natural selection in the organism's usual habitat.

When an organism finds itself in a different habitat the components of its fertilization system may evolve under directional selection 'when an effectively small daughter population is isolated for sufficient time in a habitat different from the one in which the parental population is adapted' (Paterson 1986:63). Of course, instead of speciation, extinction of the smaller population may often be the outcome.

Species are incidental consequences of the adaptation of organisms to their environment; species are effects; they are not adaptive devices. In other words species do not evolve for a purpose. Darwin had also suggested that species were fortuitous and for this he was most unpopular (Ghiselin 1969). Darwin's vision was, however, limited to believing taxonomic species resulted from adaptive change - he saw species structurally, not genetically. There is nothing that causes (sensu Williams 1966) the diversity of life, it is an accident of natural selection. In accord with this revolutionary Darwinian philosophy (Ghiselin 1969) Paterson defines a species as '**that most inclusive population of biparental organisms which share a common fertilization system**' (Paterson 1985); the origin of species is an effect of allopatric divergence in the system of mechanisms which cause fertilization or, at least, greatly increase the probability of fertilization occurring. This is the core of Paterson's Recognition Concept of species.

2.4 Classification of the D. nasuta subgroup

The classification of drosophilid flies in the Drosophila nasuta subgroup is based upon similarity. They are named in a way which is logically consistent with the Linnaean hierarchical scheme; a classification based on categories each of which (except the lowest) has a number of subordinate categories. Note how in the first sentence of this paragraph there is evidence of the ever-present influence of Linnaean methodology in casual biological writing: 'drosophilid' is familial rank, 'flies' are an order, Drosophila is a genus and nasuta a species-group name. In the Linnaean system organisms

are ranked according to their morphological similarities. The genus Drosophila has several species groups (not one of Linnaeus' original ranks); one of these - the immigrans species group - has six subgroups, one of these is the nasuta subgroup. Within that subgroup there are several species complexes. In one of the complexes there are three taxonomic species D. nasuta, D. albomicans, and D. kepulauana (Wilson et al., 1969). In the other complexes of the nasuta subgroup there have been 14 taxa named and 6 others referred to by the notation 'Taxa A-F'; of these 20 about 10 are regarded as being 'good taxonomic species'. In other words, a taxonomist armed with good descriptions could identify ten morphological classes (species taxa). The number varies depending on how much 'weight' (Mayr 1969) is given to particular morphological, physiological, cytological etc. traits. A classification of these taxa is given in Appendix A. Drosophila albomicans and D. nasuta are treated as synonyms because these names refer to a single genetic species. This is already the practice for many (Sajjan and Krishnamurthy 1972, Rajasekarasetty et al. 1976, Ranganath and Krishnamurthy 1981, Hägele and Ranganath 1982) but not all (Lambert 1982, Kitagawa et al. 1982, Wakahama et al. 1983, Hatsumi and Wakahama 1986) researchers.

The present work is a study of predictions derived from the Recognition Concept. It involves hybrid populations which are made by crossing two **genetic** species. One of these genetic species is coextensive with the taxonomic species D. nasuta, the other with the taxonomic species D. kepulauana. These are cryptic species, in the sense that, when dead, they cannot be discriminated (Kitagawa et al. 1982). Were it not for population genetical or behavioural studies (i.e. evolutionary studies) these taxa would be synonymized for there are no consistent morphological differences (Wilson et al. 1969, Kitagawa et al. 1982). It has been found, for example, that kepulauana females mate very rarely with nasuta males (Spieth 1969, Kitagawa et al. 1982, and the present study), even when confined together. This evidence indicates that they do not, at least as far as this can be judged under artificial

conditions, share the same SMRS and, therefore, that they do not represent a single genetic species (sensu Paterson). They represent two distinct fields of gene recombination.

When similar mating experiments are done with flies from the Thai or Taiwanese populations and Seychelles or other Afrotropical populations, females and males mate at random. Kitagawa et al. (1982:132) found that 'The average isolation index (Levene 1949) was 0.002, -0.009 and 0.069 in crosses between D. nasuta and D. albomicans, among D. nasuta, and among D. albomicans, respectively'. Thus, mating is at random. This suggests that the populations are part of a single genetic species. Additional biogeographic evidence will be presented in the following sections which demonstrates that there is no significant geographic barrier between any of the populations in India and east Asia, as was once thought (Wilson et al. 1969). A physical break does not exist between east Asian and Afrotropical populations.

Similar behavioural studies and biogeographic information may be used to determine the degree to which the other species taxa are coextensive with other genetic species. A number of behavioural studies have been made (Wakahama, et al. 1968; Spieth 1969; Ranganath and Krishnamurthy 1975a,b; R. Wright 1977a,b; Leroy and Eucher 1981; Lambert 1982; Kitagawa et al. 1982; Takanashi 1983; Asada and Kitagawa 1983; Balwin 1983b). The results suggest that one of the principal diagnostic traits in the taxonomy of these flies - the karyotype - is highly variable within single genetic species (Fig. 1). Much has been written about the chromosomes of D. nasuta and D. sulfurigaster; the literature is reviewed in Clyde (1982), Wakahama et al. (1983), Hägele and Ranganath (1983) and Ramachandra and Ranganath (1986). It is evident that the chromosomes are highly polymorphic and should not be given great 'weight' in taxonomy. Part of the polymorphism is due to chromosomal inversion (loc.cit.). A number of studies have been made which putatively demonstrate that the species are 'closely related' by virtue of their having 'similar' banding patterns (see especially Clyde 1982, Kitagawa 1978). With such data, it is not possible, however, to infer the 'limits' of a

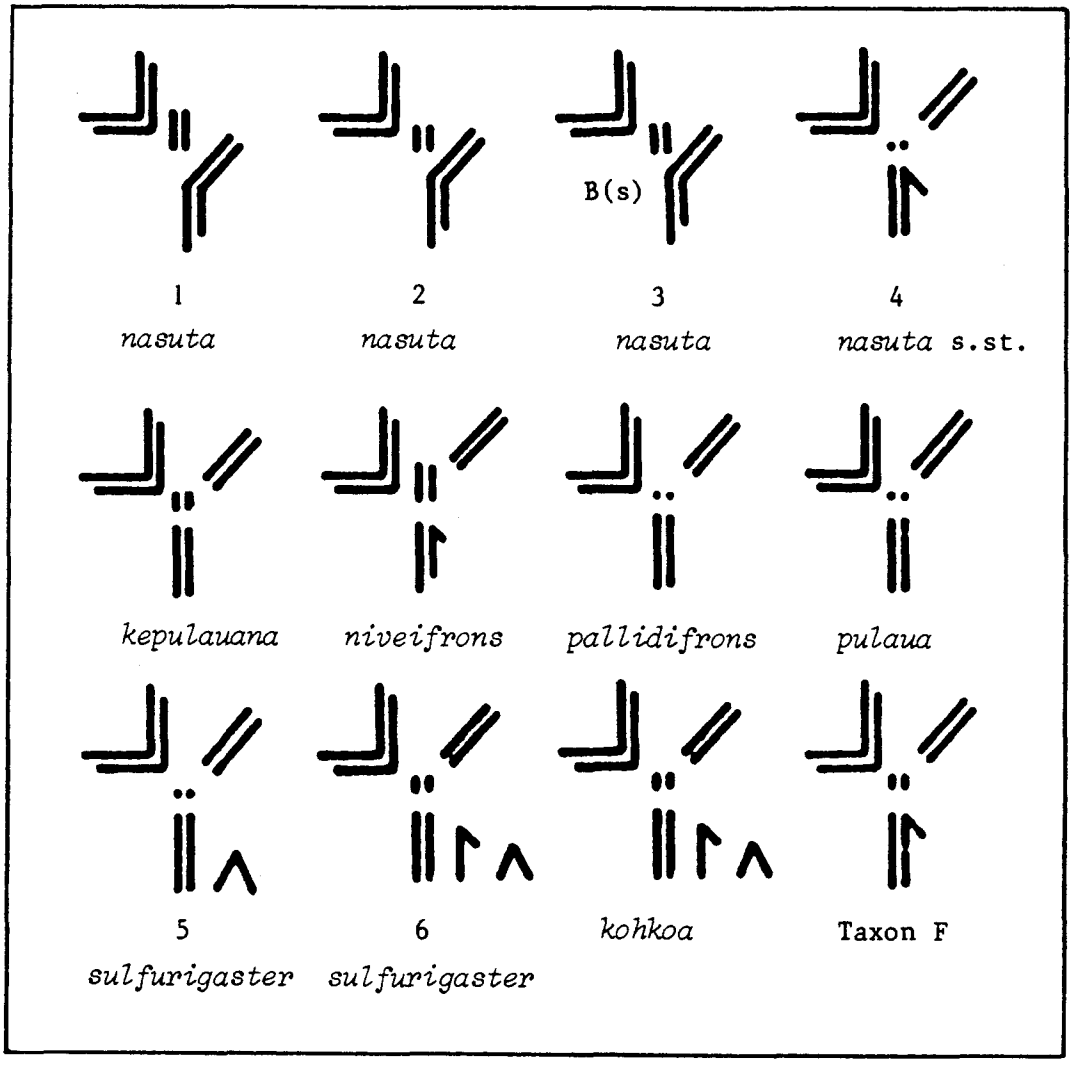


Figure 1. Metaphase chromosomes of the nasuta subgroup species (from Wakahama et al. 1983). B(s): supernumerary chromosomes sometimes found in southeast Asian populations (Kitagawa, pers.comm.); populations from 1, Japan and Taiwan; 2, 3, Thailand; 4, Seychelles Islands, Madagascar, India, Africa; 5, southeast Asia, e.g., Philippines, Borneo, Thailand; 6, Oceania (2 rods), India (J-shaped Y), New Guinea and northern Australia (V-shaped Y). Taxon F (Kitagawa 1978) resembles D. pallidifrons in external morphology and is found in southeast Asia.

genetic species (see p. 18, Lambert and Paterson 1982). Given certain assumptions about which population (or species) has the ancestral banding sequence, it is only possible to detect the direction of evolution; not the limits of the field for

gene recombination. One can use a chromosomal marker or a genetic marker to demonstrate the limits of a field of gene recombination, of course.

Table 1. The genetic species corresponding to taxa in the Drosophila nasuta subgroup. (See also Appendix A).

1. Drosophila kepulauana Wheeler, 1969
2. Drosophila kohkoa Wheeler, 1969
3. Drosophila nasuta Lamb, 1914
4. Drosophila niveifrons Okada & Carson, 1982
5. Drosophila nixifrons Tan, Hsu & Sheng, 1949
6. Drosophila pallidifrons Wheeler, 1969
7. Drosophila pulaua Wheeler, 1969
8. Drosophila sulfuriqaster (Duda), 1923

What are the genetic species which are coextensive with the species taxa of the D. nasuta subgroup? The only appropriate evidence for the existence of a genetic species is that which suggests that a group of flies are part of an inclusive population the members of which share a common fertilization system. Positive assortative mating in the 'usual habitat' is the best evidence that at least two genetic species are present. Laboratory studies can only approximate the usual or normal habitat. It is in this habitat that the SMRS evolves by natural selection and in which the sexes became coadapted (Paterson 1985). Nevertheless, to observe positive assortative mating among Drosophila under artificial conditions is good evidence that two distinct fields of gene recombination exist in nature. Table 1 is a list of the known genetic species which correspond to taxa classified in the D. nasuta subgroup; these all, with two exceptions mentioned below, have different sexual behaviour (Spieth 1969, Asada and Kitagawa 1983, McEvey 1981, Lambert 1982) and show positive assortative mating in laboratory studies (see especially Kitagawa et al. 1982). Drosophila nixifrons and D. niveifrons have not yet been adequately studied although some unpublished evidence (McEvey 1980) demonstrates that D. niveifrons and D. sulfuriqaster,

from northern Queensland, do not cross-mate when confined together. From this it is reasonable to conclude that in their usual habitat they mate in a positive assortative fashion as an effect of their having different SMRSs.

2.5 Distribution of *Drosophila nasuta*

Drosophila nasuta was discovered in 1908 on the Seychelles Islands (Lamb 1914, Fig. 2) and was not reported again for more than half a century. When Wilson *et al.* (1969) reviewed the 'systematics' of the *nasuta* subgroup, *D. nasuta* was still thought to have a very restricted distribution, not unlike those of the morphologically similar taxa *D. pallidifrons* (then known only from the Caroline Islands) and *D. pulaua* (then known only from the Semongok Forest Reserve, Sarawak). However, soon after 1969, *nasuta* records began to appear in publications concerning the drosophilid fauna of Africa and India; Wilson *et al.* (1969) had based their work principally on the fauna on the other side of the world - the southeast Asian, Australasian and Pacific Ocean Regions.

In the early 1970s *D. nasuta* was reported from Madagascar, Sri Lanka and numerous localities in India (summarized in Kitagawa *et al.* 1982). By 1979, *D. nasuta* had been found in Kenya, three more localities in India and on Reunion Island. Since 1979 *nasuta* has been found on two more islands of the Indian Ocean (Mauritius and Rodriguez Islands; Tsacas, pers.comm.); from Cameroun, Dahomey (= Benin Rep.) and Congo in equatorial Africa; from Swaziland in southern Africa (this study); from many localities in north-eastern India (e.g., Calcutta and the upper Ganga and Brahmaputra River valleys) and from the Andaman Islands in the Bay of Bengal (Clyde 1980; David and Tsacas 1981; Lambert 1977; Krishnamurthy pers.comm. to Kitagawa (Andaman Is.), cited in Kitagawa *et al.* 1982; Mather and Thongmeearkom 1978; Mather *et al.* 1974; Ranganath and Krishnamurthy 1976, 1978; Gowda *et al.* 1977; B.K. Singh pers.comm.). With respect to the Oriental range of *nasuta* (formerly called *albomicans*; see above)

additional collections, since 1969, have led to the expansion of its known range, as well. Lin et al. (1974) report that nasuta sensu lato is a dominant species in Taiwan, with population 'centres' in northern, southern and eastern parts of the island (Lin et al. 1977). In the Oriental Region the species occurs on the Ryukyu Islands through Taiwan to Thailand and at least two localities on the Malay Peninsula (Penang and Kuala Lumpur) (Kitagawa et al. 1982).

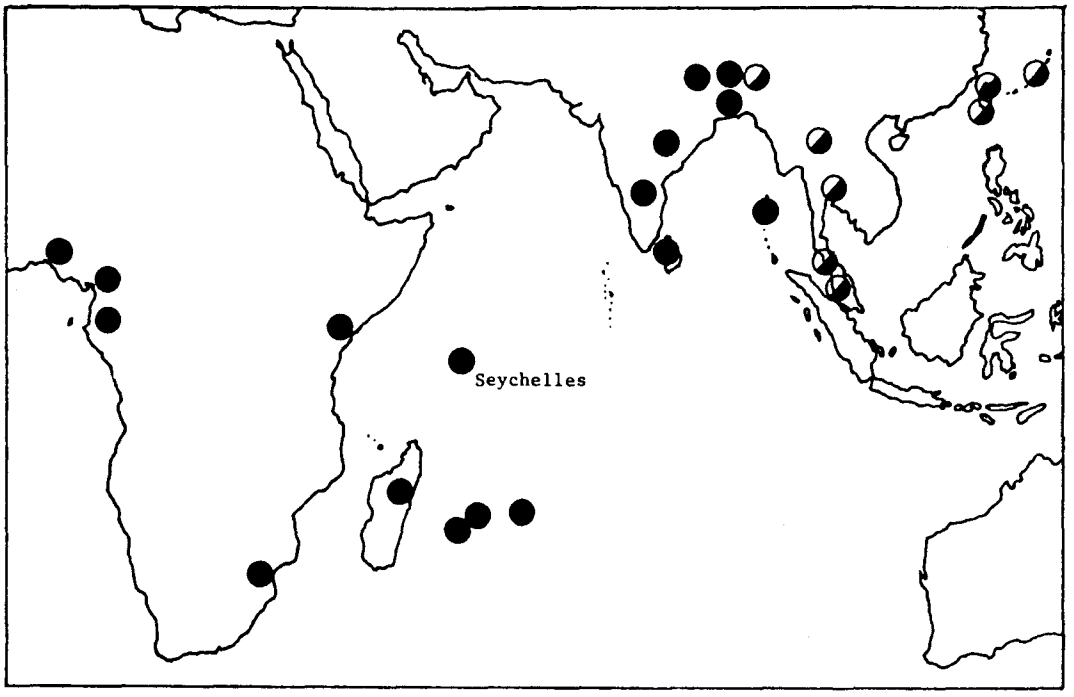


Figure 2. The distribution of Drosophila nasuta. In Africa and India the chromosome number is 2n = 8 (black circles), in east Asia the chromosome number is 2n = 6 (black and white circles). (Singh and Gupta 1979, Rajasekarasetty, et al. 1979, Wakahama and Kitagawa 1980, Ranganath and Krishnamurthy 1981, Ranganath and Hägele 1982, Hägele and Ranganath 1982).

Rather than being a remote island endemic (Wilson et al. 1969) nasuta is now known to be a very widespread species - a subcosmopolitan species. It occurs throughout Africa, India and east Asia and is common on many Indian Ocean islands. Its status as a widespread species may be recent, perhaps as recent as the beginning of trans Indian Ocean trading (David and Tsacas 1981) four to five centuries ago. But probably not as recent as the above sequence of rediscovery might suggest.

It is probable that when D. nasuta was discovered on the Seychelles it also occurred in numerous other places in the region, specifically on nearby mainlands: Madagascar and Africa. Of course, the type-locality (Seychelles) is totally independent of the locality of speciation. The type locality has no necessary connexion with the place where the species arose. Indeed it is quite possible that nasuta evolved in or near Borneo and has spread quite recently (between five and one hundred years ago) to Africa and oceanic islands. This expansion is similar to that of the 'recently' cosmopolitan species, D. simulans and D. melanogaster, which have expanded from an Afrotropical origin (Lachaise et al. 1988).

No live material was available from the Seychelles until 1971. Only then was it realised that flies from the east Asian populations and from the Seychelles mate as if conspecific (Wakahama and Kitagawa 1972, 1980, Kitagawa et al. 1982). However, apparently because of ill-defined species concepts, most workers continue to treat D. albomicans and D. nasuta as different genetic species. They base this on the lack of karyotypic and allozymic similarity and on rather dubious biogeography. However, it is possible to interpret the variability in chromosome number, and in allozymes, in certain cases (e.g., Thai and Indian populations of D. nasuta) as an intraspecific phenomenon (Oakeshott et al. 1981) and to argue that populations are reproductively continuous.

B.K. Singh (pers.comm. 1986) has collected nasuta at many localities in northern India and reports that it is a very common species wherever Drosophila are found. At high altitudes (e.g., in the Khasi Hills along the Brahmaputra River) he has found populations characterized by the 2n = 6 karyotype. At lowland localities along the Ganga and Brahmaputra Rivers the 2n = 8 karyotype is found. It is evident, then, that the populations which have 2n = 6 and 2n = 8 karyotypes are not significantly geographically isolated from each other (Singh and Gupta 1979). They do, in fact come very close together (Fig. 2) and are not separated by some '3000 miles' (Wilson et al. 1969). This means that populations of nasuta are continuous across southern Asia to the Far East.

The karyotypic difference is superficially clear-cut. But supernumerary chromosomes have been found in populations from northwest Thailand (Hatsumi and Kitagawa 1980). The diploid number for D. nasuta is sometimes 2n = 6 (or 2n = 6 + 2B) and sometimes 2n = 8. Ramachandra and Ranganath (1986) discuss the outcome of hybridization between the two main karyotypic types. After a cross between the 2n = 8 male and 2n = 6 female, karyotypic mosaicism occurs for about 15 to 20 generations; strains of this type they call 'Cytorace I'. From F_{20} onwards the karyotype became stable, in Cytorace I, with males and females having 'different diploid numbers of chromosomes and this condition breeds true' (Ramachandra and Ranganath 1986). Cytorace II, is produced by the reciprocal cross and is 'karyotypically stabilized ... with males 2n = 6 and females 2n = 6' from F_{20} onwards.

At Kuala Lumpur nasuta populations are separated from the nearest known locality of kepulauana (Kota Tinggi, Fig. 3) by 290 km of scattered plantations and Malaysian jungle in which no Drosophila collections have been made (as far as can be determined). This is where it might be possible to find both nasuta and kepulauana.

2.6 Distribution of Drosophila kepulauana

In contrast to nasuta the distribution of kepulauana is restricted. It has been reported from only four places (Wilson et al. 1969, Kitagawa et al. 1982). The type locality is Manilos in Brunei, Borneo (M, Fig. 3). The second locality (Miri) is about 50km across the border to Sarawak. The third locality (Panitan, southwest end of Palawan Island, Philippines) is also relatively close; the most significant geographical barrier between Panitan and Brunei is the 50km wide Balabac Strait. The final record (Lambert 1976) is far away (1200 km) at Kota Tinggi, Malaysia (Fig. 3). On the basis of negative results from two collecting expeditions, Kitagawa (pers.comm.) doubts the latter record but notes that this distribution matches the biogeographic pattern of D. pulaua

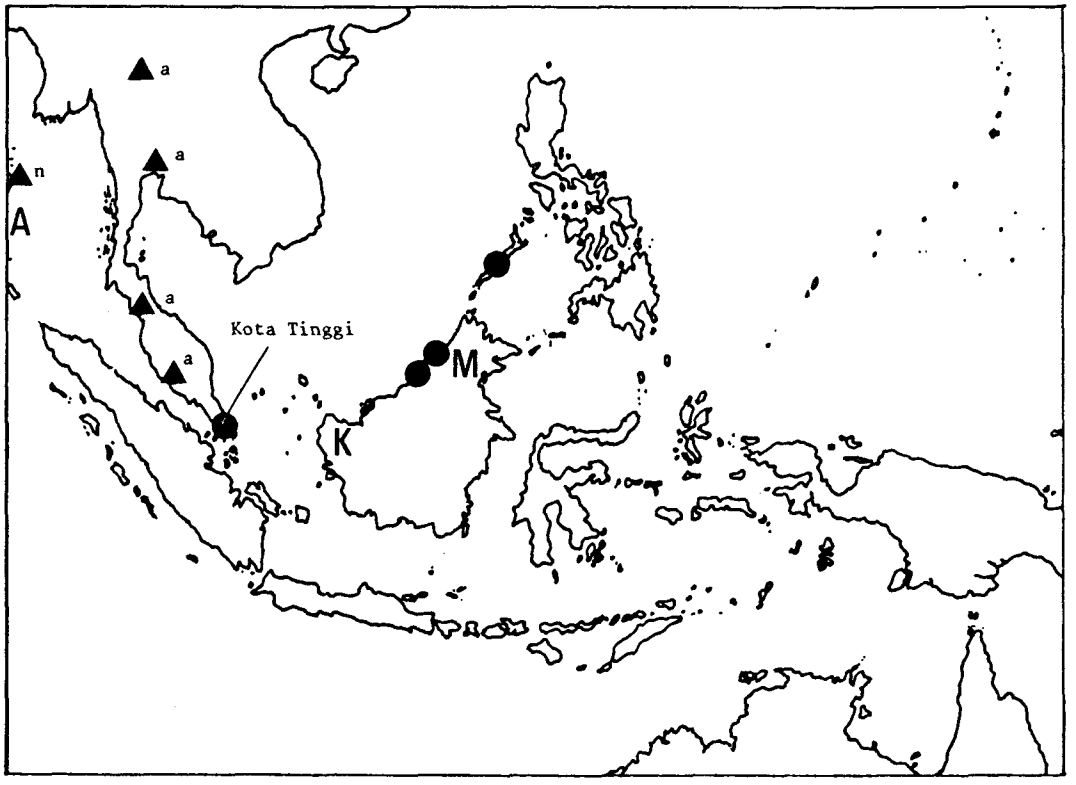


Figure 3. Distribution of Drosophila kepulauana (circles). The closest known localities of Drosophila nasuta (triangles) are also shown. Abbreviations: n, 2n = 8 karyotype of nasuta; a, 2n = 6 karyotype of nasuta; A, Andaman Islands; K, Kuching; M, Manilos.

(Fig. 4) which occasionally 'turns up' on the Malay Peninsula while it is normally found only in Borneo. The western limits of the distribution are thus uncertain; this is unfortunate because this is where the range is adjacent to, or overlapping with, that of nasuta. It is certain, however, that populations of kepulauana are not well established outside Borneo.

Kuching (western Sarawak; K, Fig. 3) lies between Kota Tinggi and Brunei; it has been visited twice by Japanese collectors (Wakahama, Kitagawa, Watanabe, Hihara and Fuyama in 1971 and 1979; cited in Kitagawa et al. 1982 and Kitagawa pers.comm.) and, although five other species of the nasuta subgroup were collected, there are no reports of kepulauana. This is puzzling and Kuching is therefore another area, apart from the southern Malay Peninsula, where further intensive

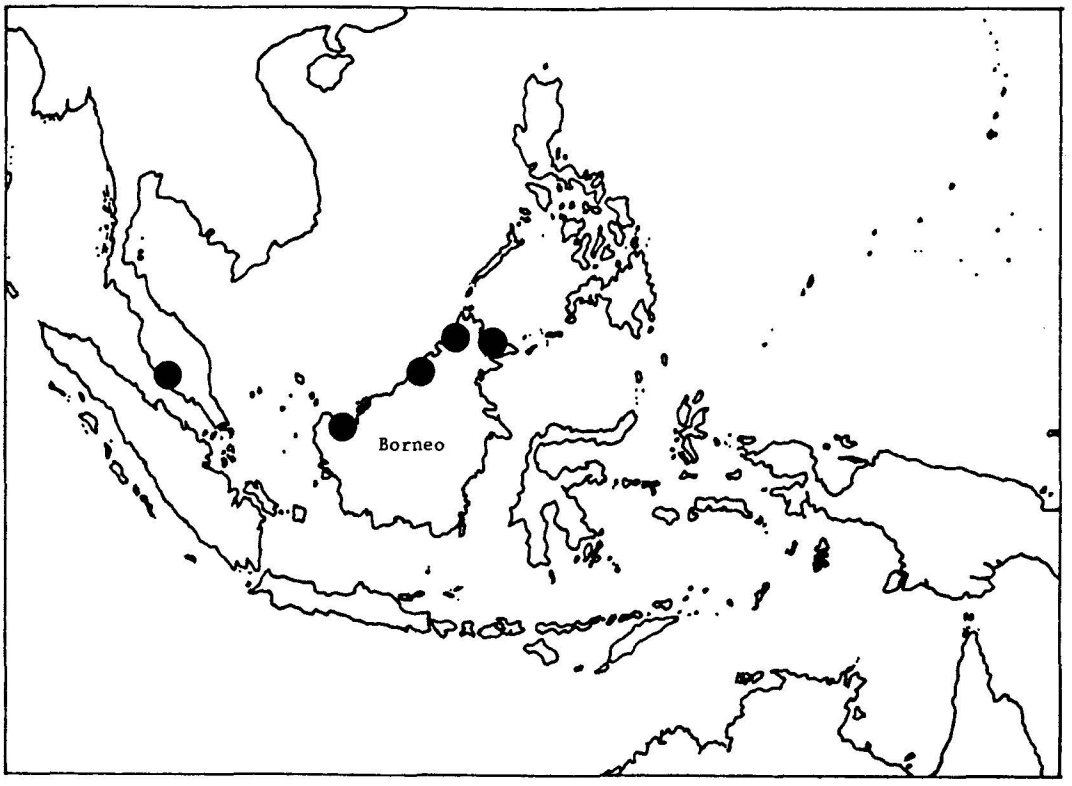


Figure 4. Distribution of Drosophila pulaua.

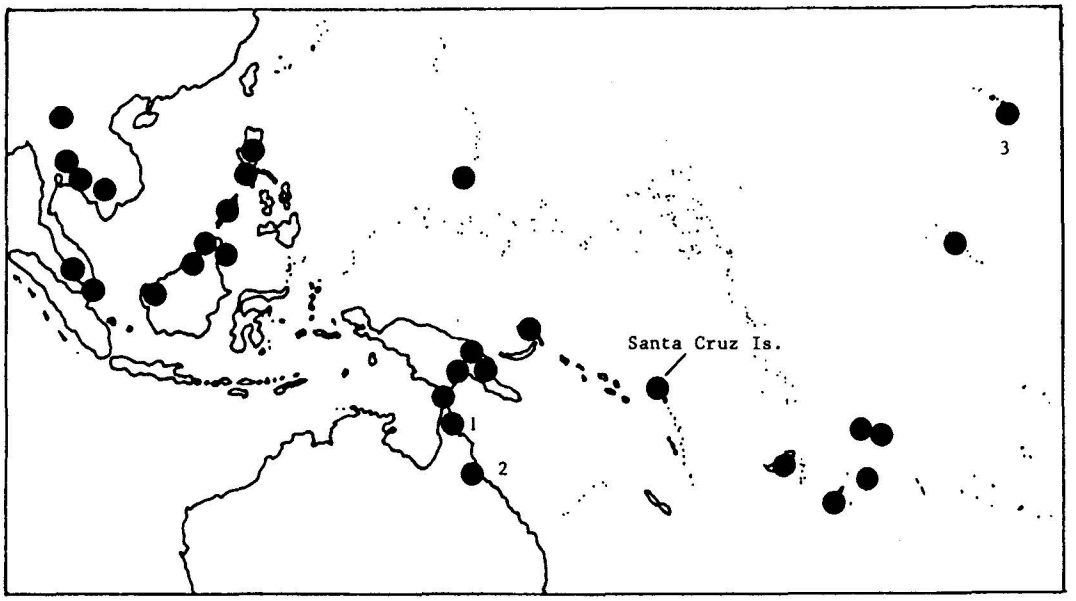


Figure 5. Distribution of Drosophila sulfuriqaster. 1, Iron Range; 2, Cairns; 3, Hawaii.

collecting may throw some light on the extent of the ranges of nasuta and kepulauana. The area just north of Singapore (corresponding to the closest potential natural contact between nasuta and kepulauana) is largely deforested in the lowlands with remnant montane forests and gallery forests along rivers. In other words it is an ineffective barrier to occasional dispersal.

2.7 Biogeography of the Drosophila nasuta subgroup

In 1908 when Drosophila nasuta was discovered on the Seychelles Islands in the central Indian Ocean (Lamb 1914), morphologically similar flies were unknown (Lamb 1914). D. sulfuriqaster was first reported in the 1920's (Duda 1923) from southeast Asia and D. nixifrons was described in 1949 from central China. An extensive survey of islands of the Pacific Ocean in the 1960's (Wilson et al. 1969) yielded a number of previously unknown, but apparently 'closely related' (Wilson et al. 1969) species. Several appeared to be restricted to oceanic islands but one (D. pallidifrons) is now known to be widespread (Kitagawa pers.comm.). The most recent addition to the group, D. niveifrons, resulted from collections in northern Australia and New Guinea (McEvey 1981, McEvey and Bock 1982, Okada and Carson 1982) and further species may still await discovery in the remote forests of Irian Jaya and Java which have not been surveyed to the same extent.

Most of the genetic species (Table 1) are found in localities around Borneo in southeast Asia. Drosophila sulfuriqaster is generally the most common species in that region and southeast of Borneo, in New Guinea, northern Australia and on islands of the Pacific (Fig. 5). In the other direction (westwards and northwards from Borneo) nasuta has dispersed widely. All remaining species are thought to have relatively restricted distributions; mostly in the vicinity of Borneo.

With respect to the cytological variability of sulfuri-

gaster (Fig. 1), it is important to note that the designation of three subspecies by Wheeler (in Wilson et al. 1969), does not correspond to three mating types found by Spieth (1969) in the same species, even though the letters 'A', 'B' and 'C' are used in both studies which are printed one after the other. For example, in the domain of one of the subspecies (A), all three 'mating-types' (A, B and C) are found (Fig. 6). There is no evidence that the variability in the degree to which the males wing is extended, which, incidentally, has not been reported again (Lambert pers.comm.), is associated with different responses in females. If the variability were not within the sensibilities of females, one might predict that natural selection would favour a less variable or more coadapted situation.

Kitagawa et al. (1982) have collected six species (kepulauana, sulfuriqaster, pulaua, kohkoa and a species resembling pallidifrons) from Miri on Borneo (near Manilos; M, Fig. 3) and conclude that Borneo must be near the place of origin of these species and that 'there has since been dispersal eastward to Hawaii and westward to Africa, with further speciation occurring along the way' (1982:120). The genetic species corresponding to the nasuta subgroup are believed to be phylogenetically closely related to the genetic species coincident with the immigrans species group (Wilson et al. 1969) and 'it appears that the origin of the immigrans species group was in Borneo' (Wilson et al. 1969, Throckmorton 1975, Kitagawa et al. 1982, Wakahama et al. 1983).

It is suggested by Wakahama and Kitagawa (1972) and Kitagawa et al. (1982) that nasuta has dispersed to India, Africa, and islands of the Indian Ocean. On the basis of morphology, hybridization data, karyotype similarity and geographical distribution Wakahama et al. (1983:315) further conclude that Thai populations of nasuta later gave rise to the nasuta populations which differ in chromosome number and are now found to the north east (e.g., Vietnam, Taiwan, Japan). Adaptation to cooler habitats (e.g., higher latitude and altitude; B.K. Singh pers.comm.; Lin et al. 1977) has possibly resulted in certain physical differences (viz.

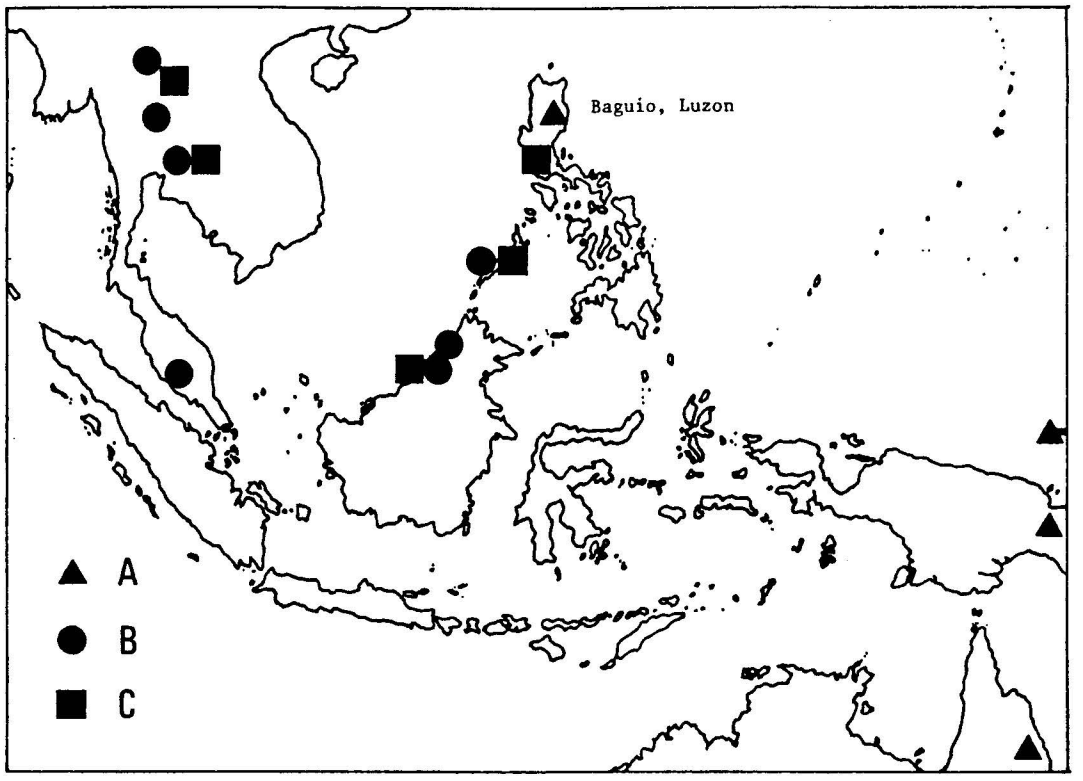


Figure 6. Distribution of three Drosophila sulfuriqaster mating types, designated A, B and C by Spieth (1969). Apart from the Australasian records this distribution corresponds to that of one of the three subspecies (Wheeler 1981) designated 'A' D. s. albostriqata by Wilson et al. (1969).

chromosome number) but the SMRS has remained unchanged. The SMRS of a widespread and polymorphic species is likely to remain unchanged while populations remain large and panmictic (Henderson and Lambert 1982). Physical differences may be the result of selection in different local conditions (Henderson and Lambert 1982, Oakeshott et al. 1981).

The question of whether natural hybridization occurs between nasuta and kepulauana is of some interest in the present study. However, as far as is known their distributions do not overlap and therefore the opportunity for natural hybridization does not exist; no natural hybrids have been found. Even if the distributions did overlap, hybridization may not necessarily occur given their reluctance to interbreed (in one direction) in the laboratory (Kitagawa et al. 1982 and

this study). No thorough screening of the natural populations have been undertaken in the regions around Singapore and western Borneo where vagrant individuals may occasionally be swept by wind. The Japanese expeditions (mentioned above) failed to find either nasuta or kepulauana in western Borneo (K, Fig. 3).

The present study is not directly concerned with the phylogeny of kepulauana and nasuta; what is of considerable importance is that they are unequivocally two distinct genetic species, possessing different mechanisms for achieving fertilization. The controversy which has been referred to above concerns the question of whether Thai and Taiwanese populations are part of the same genetic species as is found in India and Africa (and the Seychelles); not whether the latter populations are part of D. kepulauana. Another important conclusion, from the distributional information, is that the usual habitat of Drosophila nasuta is quite unlike that of Drosophila kepulauana. The latter is restricted to dense, humid low latitude jungles, the former is subcosmopolitan and able to utilize resources under a wider variety of environmental conditions.

3. NEW TECHNIQUES AND GENERAL METHODS

3.1 Introduction

Free-ranging sexual organisms locate members of the opposite sex and often copulate with them. This curious attractiveness, as Darwin (1859) and many others have noticed, may be hindered when animals are removed from their native or normal habitat. This suggests that the mechanisms involved are adapted to the normal habitat and that without certain environmental cues, copulation is less likely to occur. The set of signals and responses which draw animals or their cells together in the usual habitat has been called the Specific-mate Recognition System (SMRS) by Paterson (1985); syngamy is the endpoint of the SMRS. The SMRS is believed to be stable in a large population in its usual habitat. This stability may be disturbed or disrupted in small populations by altering the environment or the genes or both. The present work involves an initial disturbance to the genotype. Confinement to culturing bottles after genetic reshuffling may be viewed as an environmental shift.

The objective is to observe the outcome of mixing together in a single population the genes which describe two separate SMRSs and allowing natural selection to act upon this heterogeneous gene pool over many generations in a single environment. It is predicted that sexual signals and responses will reassemble and become more coadapted between the sexes. In other words it is expected that at the beginning sexual behaviour will be diverse and maladapted between the sexes while at the end it is expected that it will be more uniform and that males and females will copulate more efficiently. If this is observed it will represent important empirical evidence of the way natural selection acts upon sexual behaviour in a population. Individuals which share similar systems of sexual signals and responses recognize each other as potential mates and are fitter. The SMRS in nature is thought to be coadapted and intrinsically stable as a consequence. This is almost an axiomatic point but few studies

have attempted to disturb the entire genetic background of the sexual signalling system, for example by interspecific hybridization, in order to demonstrate that natural selection does indeed reassemble sexual signals after they are profoundly disturbed.

The method is to use a population of hybrid individuals in which the mating systems are at the start not coadapted and then allowing natural selection to act over many generations. It will be necessary to demonstrate that after a period more individuals share the same SMRS than at the start. If a single SMRS is the final outcome among advanced hybrids, for example among F_{20}, then it would be of interest to see if it represents a new SMRS or one or other of the non-hybrid parental SMRS's. Also it is possible that if many hybrid populations are studied it will be found that the outcome is not always the same and that stochastic processes play a role at some stage.

The best way to determine whether two individuals share the same SMRS is to observe them in their natural or 'usual habitat' (sensu Paterson) and see if they copulate. Drosophila are useful in this respect because their usual habitat is not difficult to recreate in the laboratory. Under artificial conditions mating occurs freely. However, laboratory confinement may produce unnaturally dense crowding. An increase in density may alter the frequency of copulation but it does not affect the sequence of the courtship behaviour (Tompkins et al. 1982, Cobb et al. 1987). 'Density influences mating times ... [but] courtship pattern is uninfluenced by moderate crowding' (Crossley and Wallace 1987:514).

It is self-evident that if a population of Drosophila survives for many generations some or all of its members have many components of a single SMRS in common, otherwise there would be no fertilization. However, it is true that during a prolonged period of unnatural confinement individuals which have different Specific-mate Recognition Systems (e.g., D. nasuta and D. kepulauana) may copulate and this would not necessarily indicate that they share all steps in the chain of signals and responses normally culminating in fertilization.

That they do sometimes mate suggests that they share some elements in common. Two things should be noted here. First, even if the nasuta and kepulauana strains used have previously been maintained for hundreds of generations in bottles; this doesn't make the lab-bottle their 'usual habitat'. A number of reasons for this are discussed later, most importantly though, the lab-bottle is not the habitat in which the nasuta or kepulauana SMRS's evolved. Second, in a heterogeneous population the individuals which share the greatest number of signals and responses in common will mate more efficiently than other members in the same population. They will have an above average reproductive performance and will therefore be favoured by natural selection.

The interval of time from the first courtship display to copulation is a good indicator of the relative efficiency of different courtships. This rests on the assumption (see, for example, Manning 1965, Ewing 1983) that when two individuals mate quickly they are more closely coadapted sexually than two that take many hours or days and attempt repeatedly - but do not achieve - copulation. This introduces a possible difficulty - quick and slow are relative terms and it is not immediately clear what they should be related to. However, members of a single interbreeding population may be compared; comparisons are only meaningless beyond this context.

'Efficiency' is a word that becomes important here because it will be used to refer to the number of copulations per unit time. This is the same as the sense in which 'efficiency' is understood by Dunbar (1982:11). For precision it is necessary to quote a long passage in which he discusses a metaphoric definition of adaptation as 'a solution to a problem set by nature'. He writes 'The criterion term "efficient" here conveys the notion of "design for a purpose" which I take to mean that a solution can be shown to be the most effective in terms of first principles (Maynard-Smith 1978). A character is said to be "better adapted" than any of its alternatives (qua competitors) when it permits its possessor to achieve the immediate objective set by that problem **more rapidly**, or **at less cost energetically**, or with

greater frequency, or by whatever criterion is most appropriate to that problem.' (my emphasis).

A relatively inefficient SMRS in one population is 'as good' as an efficient one in another. In each population one would only expect to find the most efficient that had ever evolved therein. With respect to evolution, mating efficiency (like sterility) is important only within populations. It is worth noting that a population, even one in which it takes many days for only some of the females to be fertilized, will persist as long as fecundity is not seriously reduced in some other way, for example by a high death rate. The only requirement for population viability or persistence is that 'the intrinsic rate of natural increase' (r) remains equal to, or greater than, zero (Birch et al. 1963, Begon et al. 1986).

To score the number of copulations per unit time is an approach well known in the ethology of Drosophila (Ewing 1983). The term 'mating speed' was introduced by Manning (1961) for this concept. A problem with the scoring of mating speed has been how to know whether slow mating is due to closely coadapted but sluggish signalling or to maladapted but active signalling. Because this problem has been overcome with the new method given below the word 'efficiency' is used and specially defined. Mating efficiency combines the time a male spends delivering sexual signals with the number of female acceptances. Efficiency scores are in this sense a better measure of the coadaptedness of an SMRS than is mating speed.

Such efficiency scores are made of the sexual behaviour of F_1 hybrids and one group of F_7 hybrids in different **milieus** i.e. among different types of females. (The French word milieu will be used often, English substitutes - midst, middle, surroundings, context - are inappropriate). For example, if F_1 hybrids were placed with many kepulauana females and only a few hybrid females, I call this a kepulauana milieu (the idea of using disproportionate numbers of hybrids and non-hybrids is explained later). The relationship between 'amount of courtship' and 'number of female acceptance postures' was not determined for any of the advanced hybrid populations. A modified method (described in section 6.2 and called the Open-

Cage method) measures the number of copulations achieved in a very large relatively unrestricted space where females have ample opportunity to move away from males. They fly away from males in the open space more often than in the closed chambers used in TRACE experiments. In closed spaces males are able to pursue females because females move away slowly by walking. By contrast, in an open space where females tend to fly away, males only achieve copulation with 'fully cooperative' females for they are unable to reorient to those that decamp by 'flight evasion'. Thus, while the TRACE method yields 'true' efficiency data (i.e. amount of courtship vs success), the Open-Cage method, yields only mating speed data (number of successes vs time). But in the Open-Cages it can be assumed that only those flies which are sexually coadapted will mate because the nature of the female's negative response is more effective. Maladapted sexual behaviour will not result in many copulations in the Open-Cages and will have relatively high ratios of energy to success in the TRACE studies.

In one sense the success of a population over many generations is automatically an empirical demonstration that sexual signals are to some degree coadapted. But to show that the coadaptedness increases is a different matter. More copulations, more quickly, after many generations is evidence of a decrease in the diversity of sexual behaviour and an increase in sexual coadaptedness. 'Increase in efficiency' seems to be the best term to describe this increase in the frequency of one system of signals and responses (an SMRS) which has arisen in the behaviourally diverse environment of an early hybrid swarm.

A system of sexual signals and responses (SMRS) which works well in one population may or may not work well in another population but this is of secondary or incidental interest. Clearly what happens in one population will be independent of what happens in another and for this reason use of the terms 'ethological isolation', 'mating choice' or 'preferred mate' are meaningless in this study, quite apart from objections to certain connotations implicit in them. Nevertheless, it is useful to relate advanced hybrids to

parental species in order to see if they are as efficient as the latter.

First filial hybrids of the cross between the behaviourally different species nasuta and kepulauana are placed in different lab-bottles to make different hybrid populations. Each is given a number and each comprises the hybrid progeny of single nasuta females (sired by one or several kepulauana males). Because these hybrids are fertile they can be maintained for many generations and their sexual efficiency can be monitored. In this chapter TRACE is described. It has been designed specifically to determine whether the F_1 hybrid flies have a maladapted recognition system and to give some information about their mating success or efficiency. It gives information about the time males spend signalling to females and the number of positive, negative or neutral responses received in return. The time-lapse video and audio apparatus used in TRACE is also used in experiments (detailed in the following chapter) about activity rhythms and in the detailed analysis of the visual and acoustic aspects of sexual behaviour. Thus TRACE is treated as a general method and presented here rather than in Chapter 5 where the most extensive use is made of it.

3.2 The TRACE video method

The TRACE method allows the detailed analysis of the amount and direction of sexual behaviour in mixed groups of Drosophila nasuta, D. kepulauana and their hybrids so that the identity of each member of interacting pairs is known. Although nasuta, kepulauana and their hybrids are morphologically indistinguishable, this technique does not require the use of differential genetic or physical marks, and the identity of each fly is known exactly. The TRACE technique is a new way of identifying individuals when no physically distinguishing mark is available. It relies on the detailed analysis of a video film. This can be done manually or with computer-assistance. In the present study appropriate computer

programmes and hardware were not available, results have therefore been derived manually.

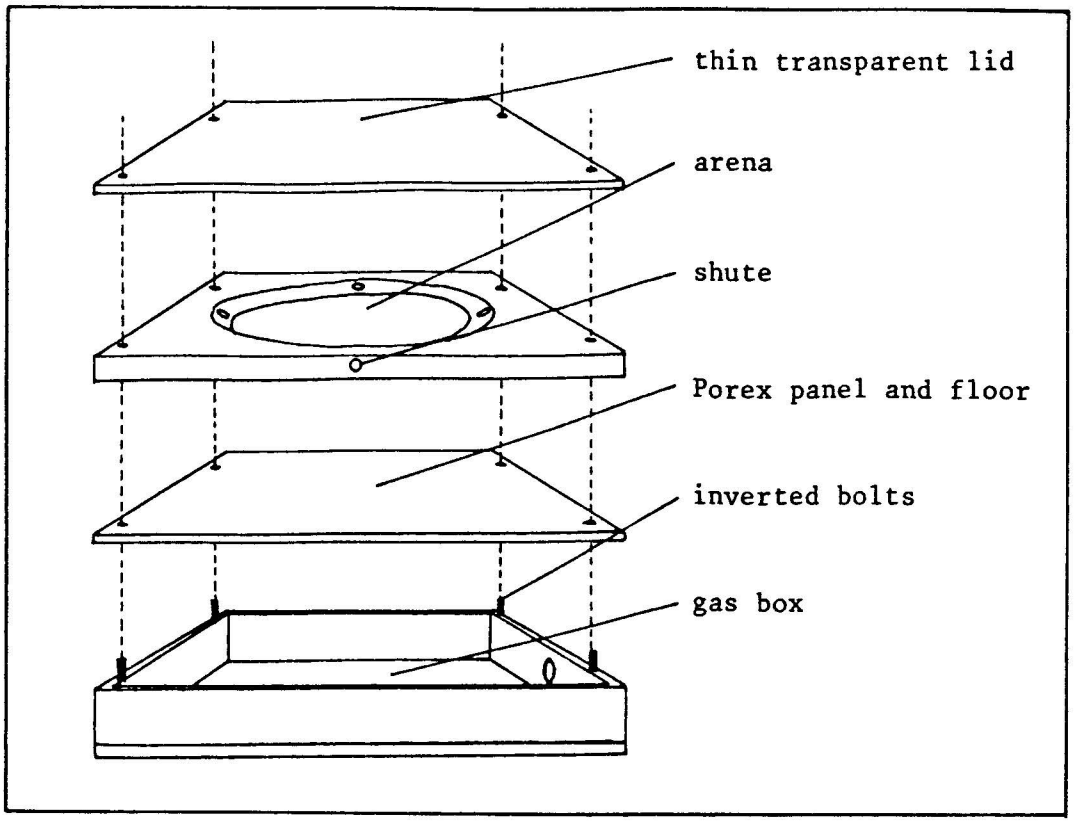


Figure 7. The fly observation arena. Diameter, 78mm; height, 5mm and volume 28cm^3.

Much information can be extracted from a 30 minute film of 30-40 flies but the most important outcome is that the ratio of time spent courting to the number of successes is calculated with precision. This is the best score of sexual efficiency and this method is the first which allows its direct calculation. It is also better than other methods which involve analysis of single pairs because it has been shown that in D. melanogaster, for example, behaviour differs when flies are in groups (Hay 1974 and other references cited by him) as is usually the case in nature. It also improves experiments in which flies are in groups of three. The usual 'choice' experiments rely on the fact that when two types of cross are possible only one and not both are effected (Manning 1965, Kaneshiro 1976); if three flies of two sexes are grouped, the first mating may be a 'male-choice' or 'female-

choice' but the second is always a 'no-choice' and thus in choice experiments the latter outcome, which is not uncommon, must be discarded.

The TRACE technique involves the following steps: (1) Flies are inserted into an 8cm-diameter Elens-Wattiaux observation chamber (Fig. 7) with a porous floor, while a camera, mounted directly above, monitors the sequence of introduction. Flies of a certain genotype, sex, species, etc. are 'labelled' by virtue of their recorded sequence of delivery into the arena. For example, type A females could be introduced 1st and 2nd and type B females 3rd and 4th (Table 2). The TRACE technique effectively identifies individuals by linking them to their 'queue-position' (01-08 Table 2) at insertion. A clear view of every fly must be maintained from the instant each is inserted until the end of the trial and for this reason 20 flies per trial is maximal, the method works best with good resolution. A digital time display is fed simultaneously to the video recorder during a trial. A 'Panasonic/National 1/2 inch Time Lapse Video Recorder Model NV-8050' with a built in 'Panasonic/National Time Date Generator, Model NV-F85' was used (Fig. 8).

(2) After the trial the film is replayed as many times as there are flies in the chamber. Each replay a different fly is tracked by continuous observation from insertion (where it is identified by a recorded commentary) until the end. Flies spend much time walking or preening; during these periods, playback can be speeded up. However, when the fly becomes sexually active, details must be noted. During a 30 min trial several hundred bouts of courtship may occur and are filmed.

(3) Three coordinates are recorded when a fly does something of interest: x (time, s), y (position, ca 1.0 cm^2 units) and z (behavior). An 'Apple-mouse' with transparent bull's-eye can be used to register a position coordinate; similarly time can be registered when a position is signalled via the 'mouse'. A programmed key-pad can record behavior. At the end of one playback one list of coordinate sets (x,y,z) - a history of times, places and activities - is available (Table 2).

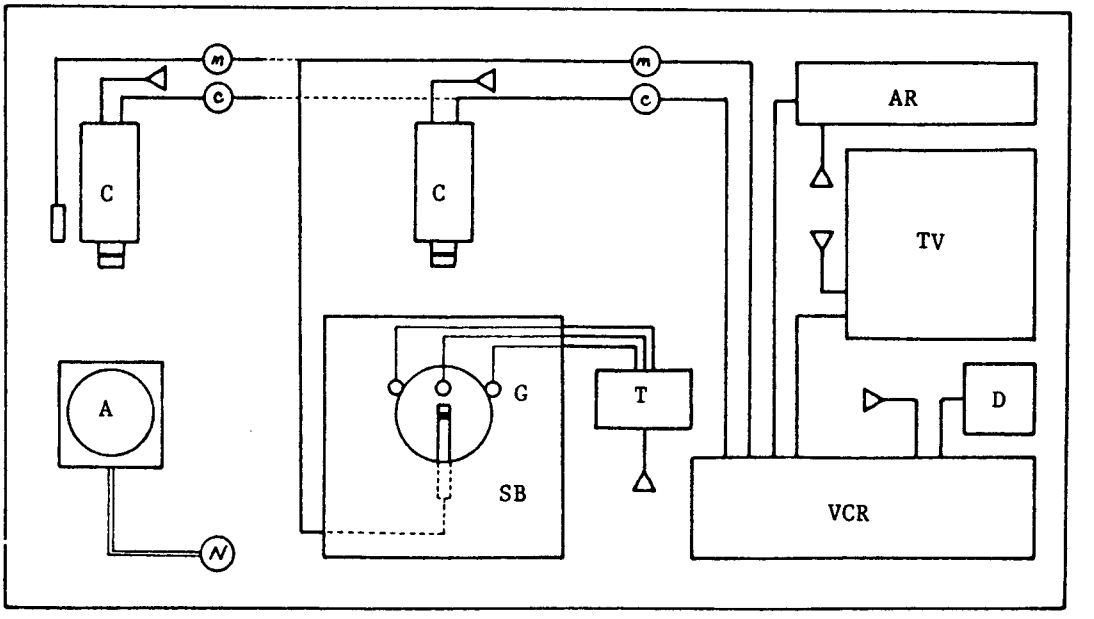


Figure 8. Scheme of video and audio recording apparatus. Two modes represented: left, for TRACE filming; centre, for audio-visual recording. A - perspex arena; AR - audio recorder, reel-to-reel; c - camera line; C - video camera; D - time/date generator; G - torch globes; m - microphone line; N - nitrogen tap; SB - sound proof box; T - voltage transformer; TV - monitor; VCR - video recorder.

(4) The identity of another fly involved in any particular sexual interaction can be determined after all flies have been processed. Points of intersection in the three-dimensional matrix of all coordinates x, y and z indicate a match. If at x-time and y-position a fly does z to another fly, then that other fly must have a matching set of co-ordinates which will be x, y and the reciprocal of z. A correct match for the z-coordinate is the reciprocal of it because if one fly is courted (z), for example, the other must be a male and he is courting (represented in Table 2 by uppercase letters). Position and time errors will occur but will rarely exceed one or two untis.

Table 2 shows hypothetical 'histories' of 8 flies during insertion and in a 5 s interval after 10 min. A computer can be programmed to search such an array for matches although

Table 2. A hypothetical TRACE data matrix. Each column is a 'history' of activity for the individuals (01-08); compare z/y in one column with Z/y in another on the same line (+/- 1 s) to find the partner. OB or ob, orientation begins; C or c, wing-extension; OE or oe, orientation terminates; i, insertion into arena; X or x, copulation; 1-8, position coordinates.

	fem	fem	fem	fem	mal	mal	mal	mal
TIME	A	A	B	B	A	B	A	B
min:s	01	02	03	04	05	06	07	08
00:01	8/i	-	-	-	-	-	-	-
00:05	-	8/i	-	-	-	-	-	-
00:10	-	-	8/i	-	-	-	-	-
00:15	-	-	-	8/i	-	-	-	-
00:20	-	-	-	-	8/i	-	-	-
:								
10:01	6/ob	-	4/c	-	4/C	6/OB	-	-
10:02	6/c	1/ob	-	2/ob	-	6/C	-	2/OB
10:03	7/c	-	-	2/c	-	7/C	1/OB	2/C
10:04	-	-	-	3/c	5/OE	-	-	3/C
10:05	7/c	1/oe	5/oe	3/x	-	7/C	1/OE	3/X
x	y/z	y/z	y/z	y/z	y/Z	y/Z	y/Z	y/Z

this was done manually. Through matching it is possible to determine which flies interacted. In the 5 s interval, male 05 courted female 03, paused for 2 s then terminated orientation; male 06 oriented to and courted female 01; male 07 oriented to - then moved away from - female 02 without courting her; male 08 oriented, then courted and mated with female 04.

During mating flies are, of course, not involved in other sexual interactions. TRACE has the additional advantage of yielding precise information about the times flies are able to be active and consequently the ratio of sexually active to inactive time can be calculated. Various aspects of sexual interaction can be readily quantified. A 20 or 30 min film yields an enormous amount of information.

From the example in Table 2 and similar much larger sets of data it is possible to determine which males courted which females, which pairs copulated, the frequency of wing extension for different males (the interburst interval or ibi, see section 4.8), the time between the start of orientation and the first wing extension, the nature of courtship-termination (i.e. whether it is a result of the female decamping or the male being interrupted etc.), the ratio of times spent passively : sexually, relative to the number of successful courtships (female acceptance postures), etc. A 20 or 30 min film yields an enormous amount of information.

3.3 Strains of D. nasuta and D. kepulauana

It is not often that hybrids can be produced between species which differ distinctively in sexual behaviour. It is also unusual to find a case where such hybrids are fertile in both sexes and can be maintained as an artificial hybrid swarm for many generations without 'breakdown'. Hybrids of the cross D. nasuta x D. kepulauana are fertile in both sexes and the two species differ in sexual behaviour. Progeny of the reciprocal cross are much more difficult to obtain. The few that were produced failed to yield offspring when crossed together, but Kitagawa et al. (1982) report some limited fertility in both sexes of such hybrids. The fertility of hybrids is discussed further in section 3.4.1.

A large number of populations were started with F_1 hybrid males and females to study the way natural selection acts on the SMRS. Unlike the study of Wallace et al. (1983), using D. persimilis and D. pseudoobscura semi-hybrid populations, the populations under study in the present work are fully hybrid in origin; non-hybrids were never introduced (cf. Wallace et al. 1983). The sexual performance of hybrids will be analysed in Chapters 5 and 6. More than 50 hybrid populations have been studied. Each has a code-number to facilitate discussion because only 26 were started in exactly the same way. They differ in age and geographic origin of the parental strains.

Table 3. List of D. nasuta and D. kepulauana strains. NDSRC, National Drosophila Species Resource Center, Bowling Green State University, USA; 'nas-' and 'albom-', D. nasuta; 'kep-', D. kepulauana; all except kep-2 and albom-1 are isofemale strains.

Code	Locality	Date	Collector	NDSRC
kep-1	Brunei, Borneo	1968	Hardy & Delf.	3122.3
kep-2	Sarawak, Borneo	-	-	3121.3-2
kep-3	kep-2 x kep-1	1985	-	-
nas-1	Swaziland	1983	McEvey	-
nas-2	Swaziland	1983	McEvey	-
nas-3	Mysore, India	1971	Krishnamurthy	3253.1
nas-4	Seychelles	1971	Kitagawa	3257.1B
nas-5	Mombasa, Kenya	1971	Kitagawa	3345.5
nas-6	Madagascar	1971	Kitagawa	3345.7
nas-7	Swaziland	1984	McEvey	-
nas-8	Swaziland	1984	McEvey	-
nas-9	Swaziland	1984	McEvey	-
nas-10	Swaziland	1984	McEvey	-
nas-11	Swaziland	1984	McEvey	-
nas-12	Swaziland	1984	McEvey	-
nas-13	Swaziland	1984	McEvey	-
nas-14	Swaziland	1984	McEvey	-
nas-15	Swaziland	1984	McEvey	-
nas-16	Swaziland	1984	McEvey	-
nas-17	Swaziland	1984	McEvey	-
nas-18	Rodriguez Is.	1985	David CNRS-BGE	-
albom-1	Thailand	-		3252.2-3
albom-2	Taiwan	1967	Throckmorton	3066.11

All strains were reared on the same food medium; the recipe was: 1.0L water, 15g agar, 150g corn meal, 55g raw sugar, 10mL moldex (1g methyl 4-hydroxybenzoate in 10mL ethanol) and 5mL propionic acid. Half pint cream bottles plugged with cotton wool were used throughout. Mites quickly contaminated bucket-cages within two to three weeks; attempts to maintain hybrid populations in them were abandoned.

Table 4. List of D. pulaua and D. sulfuriqaster strains. Eye-colours: +, wild-type; b, bright red; N53, Kitagawa's strain from Wau, New Guinea; Qld, Queensland, Australia; pul, D. pulaua; s.ab, s.bm, sulf, the three sulfuriqaster subspecies (Appendix A).

Code	Locality	Date/Collector		NDSRC
pul	Sarawak, Borneo	1968 Hardy		3121.5
s.ab-2	Tagatay, Luzon	- -	+	3138.2
s.ab-3	Semongok, Borneo	- -	+	3119.2
s.bm-2	Savusavuitangga	- -	+	3065.2
s.bm-3	Guam, Pacific	- -	+	3071.6
s.bm-4	Fiji	- -		3063.2
sulf-1	Iron Range, Qld	1981 McEvey	+	-
sulf-2	Iron Range, Qld	1981 McEvey	+	-
sulf-3	sulf-1 x N53	- -	+	-
sulf-4	Cairns, Qld	1955 Mather	b	2372.16
sulf-5	Kavieng, PNG	- -	b	3017.4

Over the several years of this study a constant light, temperature and humidity regime could not be maintained within narrow limits because of ventilation malfunction and break-downs. Each fluctuation simultaneously affected all strains and populations, presumably to the same extent. Nevertheless, prior to each behavioural assay or video-audio analysis conditions were maintained at 21.5-24.5°C with a relative humidity of 60 - 70% and a light:dark regime of 12:12 hours for at least three weeks (for ten weeks in the case of the circadian rhythm experiment, section 4.3).

With standard culturing conditions species of the D. nasuta subgroup do not breed as readily as species of the D. melanogaster species group. Indeed many species-cultures and hybrid populations were lost during the course of this study apparently as the result of air-conditioning malfunction and/or rapid medium-deterioration. More than 15 different recipes were tried with the final choice (described above) still falling short of an optimal medium (no other labs in southern Africa had good recipes). Unless flies oviposited within the

first 48 h, medium condition deteriorated in bottles and became sticky and wet in vials. This was a major problem because, of course, flies must be in good condition during an analysis of their sexual efficiency. Nevertheless, it is true that the hybrid populations were exposed to harsh conditions of temperature and fungus and bacteria infestations.

These technical and breeding problems made it necessary to manipulate cultures regularly in order to maintain healthy and large populations. This was time-consuming since cooking, bottle preparation, culturing and insectary maintenance were tasks all undertaken by the author alone. Medium and insectary problems limited the number of replicates of some experiments (particularly of the reproductive biology studies outlined in the following chapter).

3.4 Hybrid populations

Details of the fertility of inter- and intraspecific hybrids of various related species in the nasuta subgroup are given in this section. In section 3.4.2 the procedure for setting up hybrid populations is given. Since many were studied and not all were started in the same way they were numbered (Tables 6 and 7). The nasuta/kepulauana hybrids and parentals are morphologically indistinguishable and no mutant strains, to use as markers, are known. However, some interesting mutants were discovered in D. sulfurigaster late in this study but no time was available to use them in population experiments. The results of some simple crosses between these mutants are included here because the pattern of inheritance was abnormal and was probably due to hybrid dysgenesis. Some evolutionists believe that this phenomenon is relevant to models linking natural hybridization, negative heterosis and speciation. But this remains poorly substantiated. The spread of transposable elements and the potential for hybrid dysgenesis throughout a population is nevertheless of considerable interest.

3.4.1 Hybrid fertility and sterility

Hybridization is not uncommon in Drosophila. Bock (1984) goes so far as to suggest that it is more the rule than the exception. The fertility of hybrids varies greatly, however, and among the species of the nasuta subgroup there are all possible classes of hybrid fertility and sterility in interspecific and even intraspecific crosses. Table 5 shows this great variability. It can be seen in the table that D. nasuta and D. kepulauana produced fully fertile hybrids in one direction but sterile male hybrids when the parent female was kepulauana. Kitagawa et al. (1982) report fertile hybrid males from this cross but none were obtained in this study. The sperm-storage organs of some F_1 hybrid females (nasuta/kepulauana) were examined after the females had been with F_1 hybrid males for between 6 and 13 days; sperm was detected in some of them. From this evidence it can be suggested that the sterility was due to a post-insemination phenomenon. Hybrids of a cross between two other species - D. pulaua and D. sulfuriqaster - yield fertile progeny in one direction. But the latter two species differ less than nasuta and kepulauana in behaviour, as far as can be seen. Thus, for the present purposes they were less suitable. The remaining results are a summary of earlier work (Wilson et al. 1969, Sajjan and Krishnamurthy 1973-1974, Kitagawa et al. 1982). Apart from the above exception, crosses which have been repeated during the course of the present study confirm previous findings.

Hybrid fertility in both sexes seems to be exceptional and there are indeed few instances known where interspecific animal hybrids are fully fertile. This conclusion may be incorrect because only among insects like drosophilids, which may be easily manipulated, is it convenient to look for examples. The potential to produce fertile hybrids may be more general in the animal kingdom than is currently thought.

The following Drosophila species produce fertile F1 adults of both sexes (Bock 1984): bocainensis and parabocainensis (Carson 1954); arizonensis and mojavensis (Wasserman 1982); melanopalpa and canapalpa or neorepleta

Table 5. Results of inter- and intraspecific crosses in the nasuta subgroup. Data from Wilson et al. (1969), Sajjan and Krishnamurthy (1973-1974), Kitagawa et al. (1982) and the present study. Strains of one species from different geographical regions are numbered: (1) Thailand - Taiwan; (2) Africa - Indian Ocean islands; (3) & (4) India; (5) southeast Asia; (6) Hawaii and islands of the southern Pacific Ocean; (7) northern Australia - New Guinea.

		MALES											
		kok	txF	kep	(1)	(2)	(3)	(4)	(5)	(6)	(7)	pul	pal
FEMALES													
kohkoa	kok		F	f	F	o	o	o	f	o	f	f	o
Taxon-F	txF	F		F	o	o	o	o	o	o	o	o	o
kepulauana	kep	f	o		F	F	F	o	f	o	f	o	o
nasuta	(1)	f	f	**FF**		**FF**	**FF**	o	f	f	f	f	o
nasuta	(2)	o	o	**FF**	**FF**		**FF**	o	o	o	f	o	o
nasuta	(3)	o	o	**FF**	**FF**	**FF**		o	o	F	F	o	o
sulfuriqaster	(4)	o	o	o	o	o	o		**FF**	F	f	F	o
sulfuriqaster	(5)	f	o	f	F	o	o	**FF**		**FF**	**FF**	**FF**	o
sulfuriqaster	(6)	o	F	o	F	o	o	F	**FF**		**FF**	**FF**	f
sulfuriqaster	(7)	o	f	f	F	o	o	f	**FF**	**FF**		**FF**	f
pulaua	pul	f	o	o	f	o	o	F	o	o	o		f
pallidifrons	pal	o	o	o	o	o	o	o	o	f	f	f	

FF : hybrids fully fertile (both sexes)
F : F1 hybrid males sterile, F1 hybrid females fertile
f : hybrids produced but sterile in both sexes
o : no adult hybrids produced

(Wasserman 1982); americana and montana, novamexicana or virilis, plus seven other pairwise combinations of species in the virilis species group (Throckmorton 1982); two species pairs in the tripunctata species group (Patterson 1957); six species pairs in the quinaria species group (Patterson and Stone 1952); bostrycha and grimshawi and heteroneura and silvestris in Hawaii (Yang and Wheeler 1969, Val 1977, Craddock 1974); ananassae and pallidosa and three other pairs

in the melanogaster species group (Futch 1973, Ayala 1965); three pairs in the saltans species group (Bicudo 1973 cited in Bock 1984); and only azteca and tolteca in the obscura species group - but not persimilis and pseudoobscura (Bock 1984, Mayr and Dobzhansky 1945).

Some important behavioural studies have been made of D. persimilis, D. pseudoobscura and their hybrids (e.g., Wallace et al. 1983) but distinctive differences in the sexual behaviour are difficult to detect in the laboratory although they mate in a positive assortative fashion (Mayr and Dobzhansky 1945). Lancefield (1929) showed that F_1 males obtained from the cross D. pseudoobscura females and D. persimilis males, or from the reciprocal cross, are sterile, while both classes of F_1 females are fertile. They were, therefore, not suitable for the type of study undertaken here. Hybrid progeny of D. melanogaster x D. simulans are either sterile females or sterile males (Lemeunier and Ashburner 1976). Of the species pairs listed above which produce fertile interspecific progeny, none were available for study. However, close to Johannesburg, in Swaziland, a natural population of D. nasuta was discovered and D. kepulauana could be obtained from the National Drosophila Species Resource Center (NDSRC); these species also have elaborate signalling systems. For this reason they were well suited for study.

Among different strains of D. sulfurigaster many intra-specific crosses either failed altogether or produced very few offspring. An examination of the ovaries of F_1 females indicated that bilateral and complete atrophy of these organs was probably the cause (or one of the causes) for their reduced fertility.

Adult hybrids were usually larger than parental flies. This was probably due to the reduced number of them in culture vials since once hybrid numbers increased to the levels of non-hybrids (for example after several generations) they were the same size. Of course the size-difference may also be due to some heterotic effect. Do they court more actively than non-hybrids? (see section 5.3.4).

3.4.2 Formation, Maintenance and Code-numbers

This section describes in detail how hybrid populations were started, which strains of nasuta and kepulauana were used, and how the populations were handled. Additional information is included in the tables (under 'INDEX') and is relevant in Chapter 6, it is included here for convenience.

In order to force hybridization, 3-5 males of kepulauana were confined in vials with single virgin nasuta females. The reciprocal cross was attempted hundreds of times but F_1 progeny, when they were obtained, were always intersterile. Progeny resulting (i.e. 'families' of F_1 siblings) from the nasuta by kepulauana crosses were placed in bottles, each having a population number (Tables 6 and 7). Each bottle was treated as a separate hybrid population and was cultured with particular care to avoid accidental contamination by stray non-hybrids or hybrids which have escaped from other bottles. (A check of this was based on some circumstantial evidence: many strains of melanogaster were maintained and handled in the same insectary in which cultures of hybrid populations were kept, but never was there evidence of cross contamination; melanogaster has a much higher fitness under the present conditions and would quickly 'swamp' a nasuta, kepulauana or nasuta/kepulauana hybrid bottle culture). Some additional populations of hybrids of D. nasuta from Swaziland, the Pescadores and Thailand (66 to 69, Table 7) or of D. sulfurigaster from different regions (54 to 59, Table 7) were also cultured but extensive behavioural analyses were not made of these populations.

At 15-20 day intervals a random sample of between 100 and 200 flies from an old bottle were tipped into a new one with fresh food. An indiscriminate transfer at this interval meant that a mixture of old and young flies were always present together. It has also meant that no exact filial number can be given to a population. Instead the date that the populations were started has been noted (see Tables 6 and 7) and the population-ages, at which critical tests were made, have been listed.

Table 6. Hybrid population numbers (POP) and details. Populations founded from the same two isofemale strains (cf. Table 7); see Table 3 for abbreviations. Population-age in days when males were tested with kep-1 females (AGE') and nas-1 females (AGE") using the Open-Cage method; DATE, when cross made; INDEX of mating efficiency described in Chapter 6.

POP	FEMALE x MALE	DATE	AGE'	AGE"	INDEX
01	(nas-1) x (kep-1)	01.08.1983	799	813	0.00
02	(nas-1) x (kep-1)	14.05.1984	525	550	0.00
03	(nas-1) x (kep-1)	14.05.1984	526	550	0.00
04	(nas-1) x (kep-1)	27.12.1984	284	309	0.00
05	(nas-1) x (kep-1)	27.12.1984	281	314	0.00
06	(nas-1) x (kep-1)	27.12.1984	281	314	0.05
07	(nas-1) x (kep-1)	27.12.1984	281	314	0.00
08	(nas-1) x (kep-1)	27.12.1984	281	314	0.00
09	(nas-1) x (kep-1)	27.12.1984	281	315	0.20
10	(nas-1) x (kep-1)	27.12.1984	281	315	0.70
11	(nas-1) x (kep-1)	27.12.1984	281	315	0.75
12	(nas-1) x (kep-1)	27.12.1984	283	315	0.00
13	(nas-1) x (kep-1)	27.12.1984	283	308	1.00
14	(nas-1) x (kep-1)	27.12.1984	283	317	0.00
15	(nas-1) x (kep-1)	27.12.1984	283	317	0.00
16	(nas-1) x (kep-1)	27.12.1984	283	317	0.10
17	(nas-1) x (kep-1)	27.12.1984	283	317	0.05
18	(nas-1) x (kep-1)	27.12.1984	283	317	0.05
19	(nas-1) x (kep-1)	27.12.1984	284	317	0.00
20	(nas-1) x (kep-1)	27.12.1984	284	315	0.25
21	(nas-1) x (kep-1)	27.12.1984	284	308	0.00
22	(nas-1) x (kep-1)	06.04.1985	180	206	0.00
23	(nas-1) x (kep-1)	06.04.1985	-	206	
24	(nas-1) x (kep-1)	06.04.1985	180	206	0.00
25	(nas-1) x (kep-1)	06.04.1985	181	206	0.00
26	(nas-1) x (kep-1)	20.05.1985	136	161	0.00
27	(nas-1:kep-1)x(nas-1:kep-1)	15.05.1985	-	179	
28	(nas-1:kep-1)x(nas-1:kep-1)	15.05.1985	-	179	
29	(nas-1:kep-1)x(nas-1:kep-1)	15.05.1985	-	179	
30	(nas-1:kep-1)x(nas-1:kep-1)	15.05.1985	-	179	

Table 7. Hybrid population numbers and details. Populations founded from different isofemale strains (cf. Table 6). (See Table 6 for key).

POP	FEMALE x MALE	DATE	AGE'	AGE"	INDEX
31	(nas-4) x (kep-1)	21.07.1984	444	469	0.00
32	(nas-5) x (kep-1)	21.07.1984	444	470	0.00
33	(nas-6) x (kep-1)	21.07.1984	444	470	0.20
34	(nas-7) x (kep-1)	16.04.1985	175	201	0.00
35	(nas-8) x (kep-1)	21.07.1984	444	470	0.00
36	(nas-9) x (kep-1)	21.07.1984	444	470	0.00
37	(nas-10) x (kep-1)	21.07.1984	444	470	0.20
38	(nas-11) x (kep-1)	16.04.1985	175	201	0.00
39	(nas-13) x (kep-1)	21.07.1984	444	470	1.00
40	(nas-14) x (kep-1)	21.07.1984	444	470	0.00
41	(nas-15) x (kep-1)	21.07.1984	446	470	0.00
42	(nas-16) x (kep-1)	21.07.1984	446	470	0.00
43	(nas-17) x (kep-1)	21.07.1984	446	470	0.65
44	(nas-1) x (kep-2)	21.07.1984	446	470	0.00
45	(nas-2) x (kep-2)	21.07.1984	446	472	0.00
46	(nas-3) x (kep-2)	21.07.1984	446	472	0.00
47	(nas-8) x (kep-2)	21.07.1984	448	472	0.00
48	(nas-10) x (kep-2)	21.07.1984	448	472	0.55
49	(nas-12) x (kep-2)	21.07.1984	448	472	0.20
50	(nas-13) x (kep-2)	21.07.1984	448	472	0.20
51	(nas-14) x (kep-2)	21.07.1984	448	472	0.00
52	(nas-15) x (kep-2)	21.07.1984	448	473	0.00
53	(nas-17) x (kep-2)	21.07.1984	448	473	0.00

Hybrid populations 01 to 26 were replicates of a cross between the same two iso-female strains of D. kepulauana (kep-1 from Brunei, Borneo) and D. nasuta (nas-1, from Swaziland). Populations 31 to 53 were not replicates, they differ with respect to the geographical origin or iso-female strain of the parent species; 19 strains were available for these crosses, 13 of which were collected during this study in southern Africa (Table 3). All hybrid populations studied were progeny of crosses between kepulauana and female nasuta (or male

pulaua and female sulfurigaster, populations 60 to 65).

With the exception of populations 25 - 30 all populations were started with progeny from single females. Populations 25 and 26 were started by mass culturing many virgin nas-1 females with kep-1 males. Populations 27 - 30 were started with 60 kepulauana and nasuta flies (virgins) with the sexes of both species in equal ratio i.e. 15:15:15:15.

3.4.3 Genetic Markers and Hybrids

Despite the advantages of using species of the Drosophila nasuta subgroup for evolutionary studies there is one limitation - only one mutant has been reported. The phenotype, brown eye-colour, was found in D. nasuta by Wakahama and Kitagawa (1973). Kitagawa (pers.comm.) has indicated recently (1986) that some other mutants have been found in other species, no further details have been published.

When brown was described its pattern of inheritance was unknown. It was, therefore, not clear whether it could be used as a marker of heterozygous hybrids among non-hybrids as has been done in certain other Drosophila population studies (e.g., Wallace et al. 1983; Harper and Lambert 1983b). Furthermore, to use brown as a marker of heterotypes in mixed populations was problematic without a more complete understanding of its pattern of inheritance and more importantly without a second marker. Several attempts to obtain a culture of the brown mutant failed.

However, during the last year of this study genetic markers were discovered in D. sulfurigaster; unfortunately there was time only to determine their patterns of inheritance and to show that one configuration of genotypes offers some promise as a way of marking hybrids in mixed populations. Moreover, it was found that the eye-colour mutants were associated with other dysgenic phenomena, the cause of which was probably one or several families of transposable elements.

Two observations were made: (1) the iso-female strains of D. sulfurigaster, which were being maintained in culture,

varied slightly in eye-colour; two colours were discernible: wild-type red and a paler red, here called bright (b, Table 8); (2) progeny of a mass cross between two allopatric strains of D. sulfuriqaster (E, Table 8), differing in eye-colour, produced typical dysgenic symptoms in the F2 (F, Table 8). The symptoms included different eye-colour mutants: bright, dark, orange and wild-type and high frequencies of bilateral gonadal atrophy in females. Two of the type F crosses produced sexual mosaics and teratogenic mutants; providing further evidence of dysgenesis. Unfortunately the sexual mosaics were weak and it was not possible to experiment with them. In most of the mosaic individuals the orbital pollinosity, which is typical of males, was restricted to one side of the frons suggesting that the head is half male. This offers scope for exploring the association between the external anatomy and the internal neuronal organization in behaviour studies.

Table 8. Crosses between sulfuriqaster eye mutants. Eye phenotypes: (b) bright, (o) orange, (d) dark, (+) wild-type; *, many females; other abbreviations in Table 4. (NB. The eyes of males (m) and females (f) from crosses J and K were differently pigmented and were therefore recognisable in populations of non-hybrid b or o individuals.)

	FEMALES		MALE	CROSS	b	o	d	+
A	* (sulf-5)	x	(sulf-5)	b x b	1400	0	0	0
B	* (s.ab-2)	x	(s.ab-2)	+ x +	0	0	0	1300
C	* (s.ab-2)	x	(sulf-5)	+ x b	?	0	?	500+
D	* ($C.F_1$)	x	($C.F_1$)	+ x +	?	0	3	361
E	22 (sulf-5)	x	(s.ab-2)	b x +	0	0	0	1058
F	66 ($E.F_1$)	x	($E.F_1$)	+ x +	?	0	10	1509
G	17 (94.46)	x	(94.46)	o x o	0	500+	0	0
H	16 (sulf-4)	x	(sulf-4)	b x b	700+	0	0	0
I	5 (sulf-4)	x	(94.46)	b x o	0	0	0	125
J	5 (94.46)	x	(sulf-4)	o x b	0	0	165m	188f
K	5 (94.46)	x	(sulf-1)	o x +	0	0	60m	71f
L	5 (sulf-2)	x	(sulf-4)	+ x b	0	0	0	256
M	5 (sulf-4)	x	(sulf-2)	b x +	0	0	0	352

It was possible to maintain the orange-eyed mutant as a pure-breeding culture (G, Table 8). Two strains, both derived from F-type (Table 8) crosses, were apparently stable and polymorphic having an approximately equal proportion of orange-eyed individuals and dark-eyed individuals (for at least seven generations). After a year only the dark-eyed individuals were present in this strain. This indicates how the genetic cause of a phenotype may disappear from a laboratory bottle population in seven generations.

These results strongly suggest that there is a dysgenic system present in D. sulfuriqaster and it can only be guessed that such systems might also exist in the major species of this study: D. nasuta and D. kepulauana. These discoveries do not appear to be directly relevant here, however, the presence of a transposable element in a partially sympatric species which can hybridize (Table 5) is of great interest. Even though only sterile hybrids between nasuta or kepulauana and sulfuriqaster have been found in the laboratory this does not exclude the possibility that an occasional hybridization has linked the separate gene pools in nature over the long period since their phylogenetic divergence. If this were ever shown to be true it would provide an opportunity for the 'vertical migration' (cf. phylogenetic inheritance) of DNA, a very interesting possibility in any evolutionary study. In any case, it is likely that transposable elements exist in populations of nasuta and kepulauana regardless of their origin; they are known in many other species of Drosophila (Rubin 1983) and are known to pass between different - but related - species in nature (Solignac and Monnerot 1986). Some elements, particularly those causing hybrid dysgenesis, are known to decrease the reassortment of genetic variations by preventing interbreeding between individuals with certain genotypes (Rubin 1983, Bregliano and Kidwell 1983). This may be of great importance in understanding the frequency distribution of behaviour types in advanced hybrid populations.

4. REPRODUCTIVE BIOLOGY OF *D. nasuta* AND *D. kepulauana*

4.1 Introduction

This chapter deals with a number of aspects of the reproductive biology and courtship of the two species *Drosophila nasuta* and *D. kepulauana*. This information will form the basis of an investigation in Chapters 5 and 6 of the sexual interactions of their hybrids.

The word 'courtship' (cf. SMRS) has inappropriate connotations but it is convenient. It is borrowed from Old French for the solicitation of women by men and since in nature it is more often the male animal which appears to be most active during sexual interaction, courtship is used in natural history to refer to sexual displays by males to females. Morris (1956) has described it as 'the heterosexual reproductive communication system leading up to the consummatory sexual act'. I give 'courtship' a restricted meaning devoid of any connotation of sexual selection or choice or preference or anthropocentrism nor do I mean to imply that sexual interaction involves active males and passive females.

Spieth (1969) described the courtship behaviour of six species of the *Drosophila nasuta* subgroup including *D. nasuta* (Spieth called *nasuta* '*albomicans*'). McEvey and Bock (1982) referred briefly to the courtship of a seventh species which is probably *D. niveifrons* (Okada and Carson 1982). Clark (1957) made a detailed study of two subspecies of *D. sulfuriqaster* (for which he used the synonyms *D. setifemur* and *D. spinofemora*, see Appendix A) and was able to demonstrate quantitative differences in wing-beat frequency between them and their hybrids. Lambert (1982) correlated functional aspects of courtship pattern to types of pollinose and dark body markings in species of the subgroup but later (Lambert and Hughes 1984, Hughes and Lambert 1984, Lambert 1984b) began to stress the importance of structure rather than function in evolutionary studies. Asada and Kitagawa (1983) published further general observations about the courtship of *D.*

niveifrons and Leroy and Eucher (1981) gave a description of the sounds produced by males and females of D. nasuta during courtship. Nothing else has been published about the physical and acoustic aspects of the sexual behaviour of species related to D. nasuta; in particular nothing is published about the acoustic signals of D. kepulauana. No studies have been made of chemical signals in the nasuta subgroup.

Licking of the female genitalia while common in other species of Drosophila (e.g., in the melanogaster subgroup, Sturtevant 1915, Spieth 1952, Cobb et al. 1985) is a behavioural characteristic not known in the nasuta subgroup (Spieth 1969). With the exception of D. kohkoa the most important features of courtship are the circling or lunging movements of males around and in front of females (Spieth 1969). Spieth (1969) suggested that this shift away from direct contact is correlated with their light dependency and he concluded that the major stimuli are visual (Spieth 1969). However, Wright (1977b) in a study of D. kepulauana, D. nasuta and D. pulaua observed that light dependence is not obligate: 'all species were 100% fertile within nine days when kept on a 0/24 L/D cycle' (Wright 1977a:136); his earlier observation (Wright 1974 cited in Ewing 1983) of light dependency for D. pulaua is apparently incorrect.

Ewing (1983) reviewed the functional aspects of Drosophila courtship. Before him Spieth (1974) summarized extensive information about the diversity of courtship in the genus. Manning (1965) reviewed earlier work on Drosophila behaviour and was the first to initiate studies of mating speed, a concept which will be used later to categorize courtship efficiency.

The elaborate male behaviour patterns of the species of the nasuta subgroup are interesting because they involve movement to the front of the female, anterior wing-displays (except kohkoa) and no licking. Whereas in most other species of the large immigrans species group males posture behind the female and do not have such elaborate display repertoires (Spieth 1969). A possible corollary to this is that nasuta subgroup species unlike most others in the immigrans species

group have (with the exception of pallidifrons and Taxon F) dark markings and/or frontal pollinosity which would be visible to a female during courtship (Lambert 1982).

Spieth (1969:262) writes that 'Females of all stocks studied (sulfuriqaster, "albomicans", pallidifrons, kepulauana, kohkoa and pulaua) displayed qualitatively identical behaviour for both the acceptance and rejection responses.' In the present study no new female responses have been found.

The visible and acoustic aspects of the courtship of nasuta and kepulauana are examined here and some cursory studies of sulfuriqaster and other related species are presented.

4.2 Generation Lengths and Fecundity

Suppose two species of Drosophila exist together in one bottle and that they do not interbreed; at intervals of about two to three weeks start a new bottle with a random sample from the old. One could confidently predict that eventually all the flies in the bottle will be of just one type. The species which becomes extinct is the one which on average produces less progeny in two to three weeks under the set conditions. Important parameters are fecundity, time and breeding conditions. Stochastic processes may have a role too but they will diminish in importance as numbers increase.

What would be the outcome if the two species which are mixed in the bottle do interbreed? In theory two things could happen and both possibilities depend on the fecundity of hybrids. If hybrids are completely sterile the population which by chance becomes smaller will further diminish in size and go extinct. On the other hand, if introgression of the two genomes occurs, natural selection will favour the genes (or alleles) which are responsible for maximizing the mean reproductive performance of individuals and these will remain in the population while other genes or alleles become extinct or exist in a stable and reduced or altered frequency (Li

1955); the latter being dependent on pleiotropic effects.

In the hybrid populations which form the basis of this study the genes of nasuta and kepulauana are fully introgressed at the beginning; all populations are started with F_1 hybrids. Therefore it is predicted that after many generations under natural selection only the genes which lead to maximum reproductive performance will be present. What is the initial variability in fecundity of nasuta and kepulauana before introgression?

To demonstrate the variability in fecundity an analysis of the number of progeny produced by single females in a given time was made. From such data the 'generation time' or 'generation length' (the time between the eclosion of a female and the eclosion of her progeny) was determined. A different generation time between nasuta and kepulauana would make the time at which flies are transferred to new bottles a critical factor. For example if one of the species is found to have a long generation exceeding two weeks then a two week interval between bottle-transfers may very quickly cause the elimination of genes resulting in greater-than-two-week development. If prolongation of development is genetically linked to rates of sexual maturation the generation time becomes a very important factor in experiments discussed in Chapters 5 and 6.

The generation time is not, of course, exactly the same for different individuals whether they are conspecific or not. It is known, for example, that variability in generation length is caused by extraneous factors such as temperature and nutrition (Poulson 1934) acting upon larval growth.

The method for the present study is as follows: One female and several males are placed in a vial with standard medium. At this time (Day-1) the females have been adults for less than five hours. The males have been adults for four or more days. Replicate vials - 20 of kepulauana (kep-1) and 20 of nasuta (nas-1) - were run concurrently in the same culture room. Thus the same ambient temperature and light regimen applies to both species. On Day-9 the adults were removed from each vial. Each day after Day-9 the vials were examined for

adult progeny. When found they were counted and discarded. The experiment continued until Day-26. The results are presented in Tables 5 and 6 in Appendix B.

Under these conditions the minimum generation time for nasuta is 13 days and for D. kepulauana is 14 days. The difference is not great. The minimum time for nasuta is based on the eclosion of only two flies on Day-13 whereas on Day-14 a surge of 60 flies was scored in 50% of the vials. Among the kepulauana vials 29% contained adults on Day-14 (12 flies in total) whereas on Day-15 65% had adults (71 flies in total) (see Tables 5 and 6, Appendix B).

The first eggs are observed in the medium of some vials on Day-3 (both species). In the same vials the first surge of adult eclosions occurs 11 (nasuta) or 12 (kepulauana) days later. These are therefore the minimum times for the growth of eggs into adults. If these times are the median values then there should be a decline in eclosions starting on Day-20 or Day-21 which corresponds to 11 or 12 days after Day-9 when the original females were removed. No such decline is observed. This may be explained as a density dependent effect: the lower the number of larvae the greater the chance of reaching maturity and vice versa. It may also be the result of a change in the quality of the medium. The medium clearly changes during the 26 days and it may differentially favour larvae of different ages.

Many flies have developmental times (egg to adult) exceeding 11 days because large numbers of adults continue to eclose after Day-20. In fact the pattern in both species remained stable until the end of recording (Day-26). Therefore many individuals of both species have a generation length of about 20 days which comprises 17 days to complete development from egg to adult and two to three days for sexual maturation.

The variability among individuals of the same species has been found to be considerable. Standard deviations overlap extensively and are not given. The range of variability within a species is as broad as between the species.

Among the 20 kepulauana vials three became contaminated with fungi before larvae had churned the medium. Churning

usually prevents fungal growth. These vials yielded no adult progeny and were excluded from calculations. Nevertheless, this observation suggests that nasuta is better adapted to the present conditions; one would otherwise expect some of the nasuta vials to have also been contaminated.

The average total number of nasuta progeny per vial by Day-26 was 90 (s.d. = 25, n = 20 vials, 1793 flies counted) but variability is great. One female bore only 10 progeny and another 131. The average total number of adult kepulauana counted in each vial by Day-26 is 68 (s.d. = 16.3, n = 17 vials, 1150 flies counted). The maximum yield per kepulauana female was 92, the minimum 24 (excluding the three vials which failed altogether). Similarly between Day-14 and Day-26 the average number of progeny emerging per nasuta vial per day was 6.9 (n = 260) and per kepulauana vial was 5.2 (n = 221).

If nasuta and kepulauana did not hybridize and the two were placed together in a bottle, kepulauana would probably go to extinction in about 150-200 days i.e. about 10 generations. This is a very simple interpolation of the separate developmental trends observed over 26 days viz. 20 nasuta females : 17 kepulauana females on Day-0 to 1793 nasuta : 1150 kepulauana flies on Day-26. However, nasuta and kepulauana do hybridize when confined in a bottle and the above prediction cannot be tested. The results do, nevertheless, favour the conclusion that the developmental system of nasuta is more fecund under the present conditions.

The nasuta genes and/or alleles for developmental rate (as measured by generation time) and for fecundity (as measured by number of progeny per female) will probably remain in an advanced fully introgressed hybrid population. No data are available which allow comment on possible pleiotropic effects of genes for development on the one hand and sexual behaviour on the other although it would be interesting to see whether developmental rates are generally linked to sexual maturation rates. In studies of hybrid populations of D. persimilis and D. pseudoobscura Van Valen (1963) treated generation length as important because he believed that larger larvae have a 'decided advantage' in the competitive

environment of a bottle-culture.

Natural selection acts by maximizing the mean reproductive performance of individuals in a population. In a bottle-bound population of hybrids of nasuta and kepulauana natural selection will favour the individuals which complete a life-cycle in a time shorter than the time when a random sample of adults from the 'old' bottle is removed to start the 'new' bottle. If this time is about 11 days, only flies which are capable of developing from egg to adult in 11 days will be selected. Therefore, if an introgressed nasuta/kepulauana population is moved to a new bottle every 11 days the faster life-cycle of nasuta will probably prevail. In practice however, bottles are transferred about every 15 - 20 days. Thus both slow and fast developers at random will be selected to start new bottles. The transfer interval does not favour either nasuta or kepulauana. But these two species have a different fecundity in an interval of 15 - 20 days: nasuta is more fecund. Early filial hybrids are expected to have variable fecundity while only the most fecund will persist after many generations. It is predicted that advanced hybrid populations will be as fecund as nasuta.

4.3 Sexual Activity Rhythms

Knowing when flies are sexually active is obviously important in the design of experiments to study that activity. For this reason two groups of D. nasuta flies were closely monitored for 28 h to determine whether sexual activity occurs at random or in particular periods - peaks - like other species of Drosophila (Spieth 1974). If a peak (or peaks) in sexual activity is found does it correspond to the time when copulations occur? If the behaviour is found to be periodic is the 'clock' innately neuro-physiological or can external stimuli alter the rhythm? Does a male become active at an atypical time when confronted by a virgin female? If sexual activity occurs in the dark how successfully do the sexes communicate?

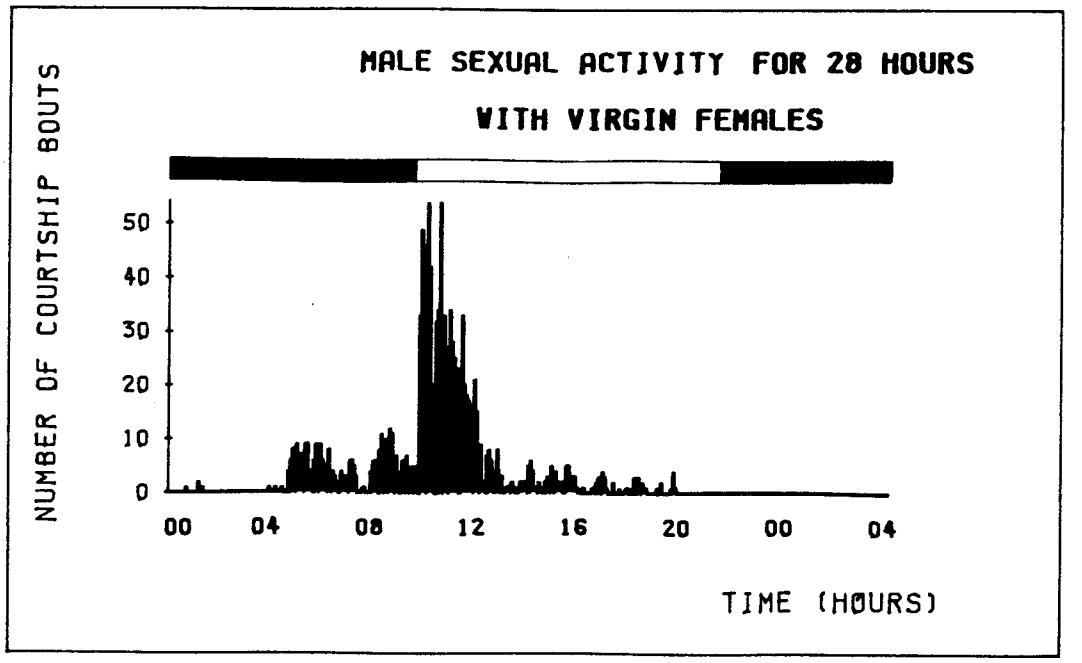
Answers to these questions will not only provide further information about the extent of variability potentially present in a hybrid population but may also make it possible to restrict observation time to limited periods of the day.

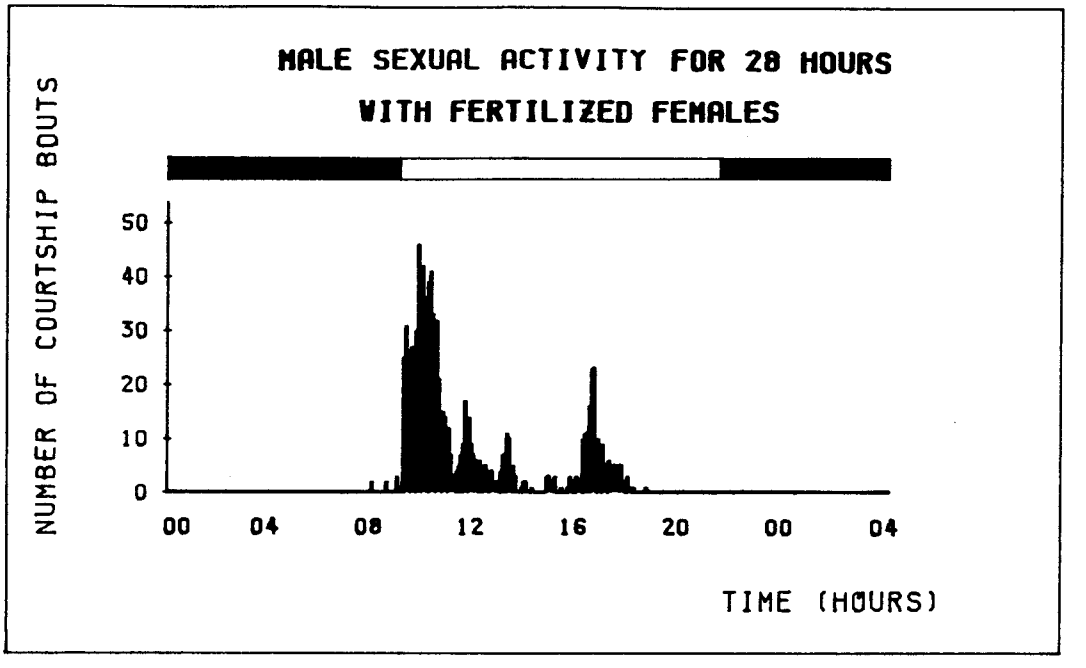
Twenty pairs of D. nasuta (nas-1, Table 3) were placed in a shallow perspex chamber (Fig. 7) beneath a video camera (C + A, Fig. 8). In a second chamber beside the first another 20 pairs were held. The two setups (Trials I and II) were filmed simultaneously for 28 h using time-lapse video. Red light in a photographic dark room was used during simulated 'night' and ordinary incandescent white light was used during simulated 'day'. In Trial I the females were 105 hour-old virgins at the start, in Trial II the females were also 105 h old but previously fertilized; in both, the males were 105 h old and had been isolated from females since eclosion. Both types of females were exposed to a minimum and equal amount of ether. During (and for four to five generations prior to) the filming, flies were maintained on a constant 12 h light : 12 h dark regimen.

Flies were introduced into the chamber 2.5 h after the commencement of the 12 h dark-phase. Thus during the first 9.5 h of the trials the flies could not see. Normally the sudden movement of an object near flies causes them to 'jump' but under red-light conditions such stimulation caused no disturbance whatsoever even when the red light source itself is waved and flashed on and off. It is therefore concluded that they have no vision in the red wavelength.

A time-lapse video system recorded behaviour continuously beginning at the time the flies were inserted into the chamber until 6.5 h after 'lights-off'. By reviewing the film at normal speed it was possible to count the number of courtship bouts and copulations in five minute intervals. A bout is when a male circles around in front of a female. A pause of more than 6 s without circling punctuates separate bouts. The results are presented as two histograms of sexual activity.

Drosophila nasuta has a sexually active phase of about two hours commencing immediately when there is light. All copulations were observed in the first 61 min in Trial I. The





Figures 9 and **10.** The sexual activity of D. nasuta over 28 h. Males with virgins (above) or with fertilized (below) females; 12 h of light (white section of horizontal bar) occurs from 09h30 to 21h30 in each trial.

number of bouts in the first 120 min of the light phase was 751, an average of 31.3 bouts each 5 min (s.d. = 11.8; n, number of 5 min intervals = 24; N, number of males = 20) in Trial I and 643, an average of 26.8 bouts each 5 min (s.d. =

10.7, n = 24, N = 20) in Trial II. Therefore in terms of the amount of courtship it appears to make little difference if the females are virgins or non-virgins. In the 60 min interval immediately after this i.e. the third hour of the 12 h light phase the average number of bouts per 5 min interval drops considerably to an average of 10.3 bouts/5 min (s.d. = 6.3, n = 12) in Trial I and to 7.7 bouts/5 min (s.d. = 4.3, n = 12) in Trial II. In Figures 9 and 10 the sexual activity patterns are inconsistent at other times of the day with the exception that during the last eight hours (seven of which were dark) no sexual signalling occurred in either trial.

In Trial I (with virgin females) 16 of the 20 females were mated in the first 61 min and no mating occurred after this. Within the first 26 min, 13 of the males (more than 50%) were in copulation. After 30 min (10h00) male activity drops but this is due to the fact that more than half of them are preoccupied with copulation. Copulations last an average of 23 min (s.d. = 3.84, number of copulations scored = 34; see Table 10, p. 80) so the increase in sexual activity which occurs some 40 min into the light phase corresponds to the termination of many copulations and the resumption of male activity. This means that mating does not retard male sexual activity. If it did there would be a decline in sexual activity directly proportional to the number of copulations but this was not observed. Rather there is a resurgence of general sexual activity reaching the peak level observed prior to most of the copulations. Despite the fact that males signalled persistently in Trial II none of the females mated again (they had mated prior to the trial).

In Trial II (Fig. 10) starting at 16h30 there is a peak of activity lasting c. 1.5 h and involving 163 bouts of courtship (20 males). A single hyperactive male is not responsible for all bouts because at least six 'courting' pairs could be identified at one time. The peak is absent in Trial I (virgin females, Fig. 9). The absence of the 'afternoon peak' in Trial I can be explained if females are least attractive soon after they have mated but progressively more attractive as time elapses. Note that in the 'afternoon'

only six hours had elapsed since the 'virgin' females mated; whereas in the other trial a much longer period of time had elapsed, at least 26 h. It can be concluded that females are not attractive soon after they have mated but that they become progressively so as time passes. Further speculation along these lines requires replication of trials since the 'afternoon peak' may be due to the chance increase of activity of a single male that excited the others.

In Trial I during the dark-phase the presence of virgin females stimulates males which are unable to see them. It is evident that vision was blocked because males could not orient nor circle 'correctly'; they probably require visual cues to maintain orientation.

Because males were sexually active in the dark only when in the presence of virgin females it can be concluded that a stimulus other than a visual one was involved. The most likely explanations are that virgin females have either a different odour, make a different sound or have - or signal through legging - a different physical condition. The fertilized females on the other hand may produce any or all of these types of stimuli but instead of exciting they inhibit males.

Three years of observation of D. kepulauana confirm that the nasuta pattern is similar. Wright (1977b) made a comparative study of the diurnal variation in the frequency of courtship behaviour of D. kepulauana, D. nasuta ('albomicans') and D. pulaua. For 24 h prior to his 30 h observation period virgin male and female flies were placed together. Thus his results are comparable with those in Fig. 10 (fertilized females). There is a good correlation between his results for nasuta and those presented here; he too found a second peak of activity during the 'afternoon' period. His results indicate that kepulauana males, like nasuta, also have a surge of sexual activity at dawn. The activity subsides during the day. However, he found no statistically significant 'afternoon peak'; his graph indicates a slight tendency only.

In conclusion it may be said that the achievement of copulation and hence fertilization requires light; at least for D. nasuta. The inference that mating is not completely

light dependent (Ewing 1983, Wright 1977b) in this and related species must be viewed with caution because if flies are crowded enough to bump into each other, light dependent stages may be dispensed with. I found no evidence suggesting that copulation is possible in darkness other than under very crowded conditions. The presence of striking silvery-white frontal pollinosity and dark pleural stripes in males and the way in which they are 'displayed' to females suggests that light plays a crucial role in the SMRS of these species (Lambert 1982).

4.4 Sexual Maturation

In section 4.2 it was observed that very young females are not attractive to males and that young males do not signal to females. It seems therefore that sexual responsiveness is dependent on age.

Sexual maturation time is the time between eclosion and copulation. It need not be the same in both sexes. Two tests which are designed to determine the minimum sexual maturation time of D. kepulauana (kep-1) are described below. In the first test males are the same age as females. In the second they are 135 h older. The flies are placed together in vials with food - five males and ten females per vial. At intervals females are removed, dissected and examined for the presence of sperm. The number inseminated is given in Table 9.

The results of the first test show that one or both of the sexes have not reached sexual maturity until between 45 and 52 h. If males have a minimum sexual maturation time exceeding females' then confinement of mature males with young females should result in earlier insemination. In the second test with mature males - 135 h older than females - females are inseminated after only 24 h. The conclusion is, therefore, that females mature earlier than males. Experiments by Wright (1977a) led him to conclude that most females (cf. 'quickest females') become receptive between 48 and 72 h after eclosion in both kepulauana and nasuta.

Table 9. Sexual maturation time of D. kepulauana. Age of males (mal.) and females (fem.); number of females inseminated per vial (no.); time after start of test = 'fem.'

mal.	fem.	no.	mal.	fem.	no.
21 h	21 h	0	>144 h	9 h	0
28	28	0	>159	24	2
45	45	0	>164	29	2
52	52	3	>169	34	6
70	70	5	>184	49	3
76	76	7	>193	58	2
94	94	9	>208	73	5
102	102	7	>208	73	2
117	117	8			

Do young males fail to mate because they do not court or because when they court their signals are not recognized? These alternatives are resolved by direct observation. In a TRACE experiment (results not given) with males of various ages it was found that 96 hour-old males courted females vigorously and 36 hour-old males did not court at all. This explains why young females were not inseminated in the first test, they were not courted.

Clark (1957:217) notes that the ovaries of the related species, D. sulfuriqaster, are immature at 24 h even though mating may occur and that 'fertilized eggs are not laid until the females are about 6 days old, the sperm being stored meanwhile in the spermathecae'.

A number of studies of D. melanoqaster and D. simulans have demonstrated that chemical signals play a fundamental role in the recognition of one sex by the other. Furthermore it has been found that ontogenetic modification of the odours of individuals is associated with their maturation after eclosion. The compounds of young melanoqaster males disappear about two days after eclosion (Antony and Jallon 1982) and are replaced by other substances peculiar to sexually mature flies. A female pheromone first appears about 30 h after eclosion and induces precopulatory behaviour in male

melanogaster (Jallon et al. 1986). There is no reason to doubt that in D. nasuta and D. kepulauana certain cuticular hydrocarbons serve as signals in the SMRS. The direct examination of such substances is beyond the scope of the present study. However, this study has indicated the presence of a 'switching effect' (Wright 1977a) which may be attributed to ontogenetic changes in cuticular substances.

4.5 Remating and Sperm Utilization

Do females remate and if so under what circumstances? How is sperm utilized by twice-mated females? If a female remates and the first and second partners have different genotypes, examination of the progeny will identify which male's sperm is utilized. If a 'series' of progeny have mixed phenotypes then it would be reasonable to conclude that sperm is mixed in the female. If progeny switch dramatically from one type to the other and never back to the original then it is possible that the sperm of the first mate has been displaced by that of the second. This hypothesis forms the basis of the four experiments described below.

To be certain that sperm displacement has occurred it must be shown that sperm depletion did not first occur; this is very difficult. Evidence that indicates the approximate time it takes for a single ejaculate to be depleted is useful.

Until the orange eye phenotype was discovered in D. sulfuriqaster (section 3.4.3) an investigation of sperm utilization was not possible in the nasuta subgroup. Efforts to obtain a stock of the brown eye-colour mutant (Wakahama and Kitagawa, 1973) were not successful and no other mutant markers have been reported.

The pattern of inheritance has been worked out for this marker (see Table 8, p. 59). It was therefore possible to use it in a test of sperm utilization: an orange female has different progeny depending on whether she is mated by an orange or by a wild-type male. The two strains used in this study are the wild-type strain sulf-1 and the orange-eyed

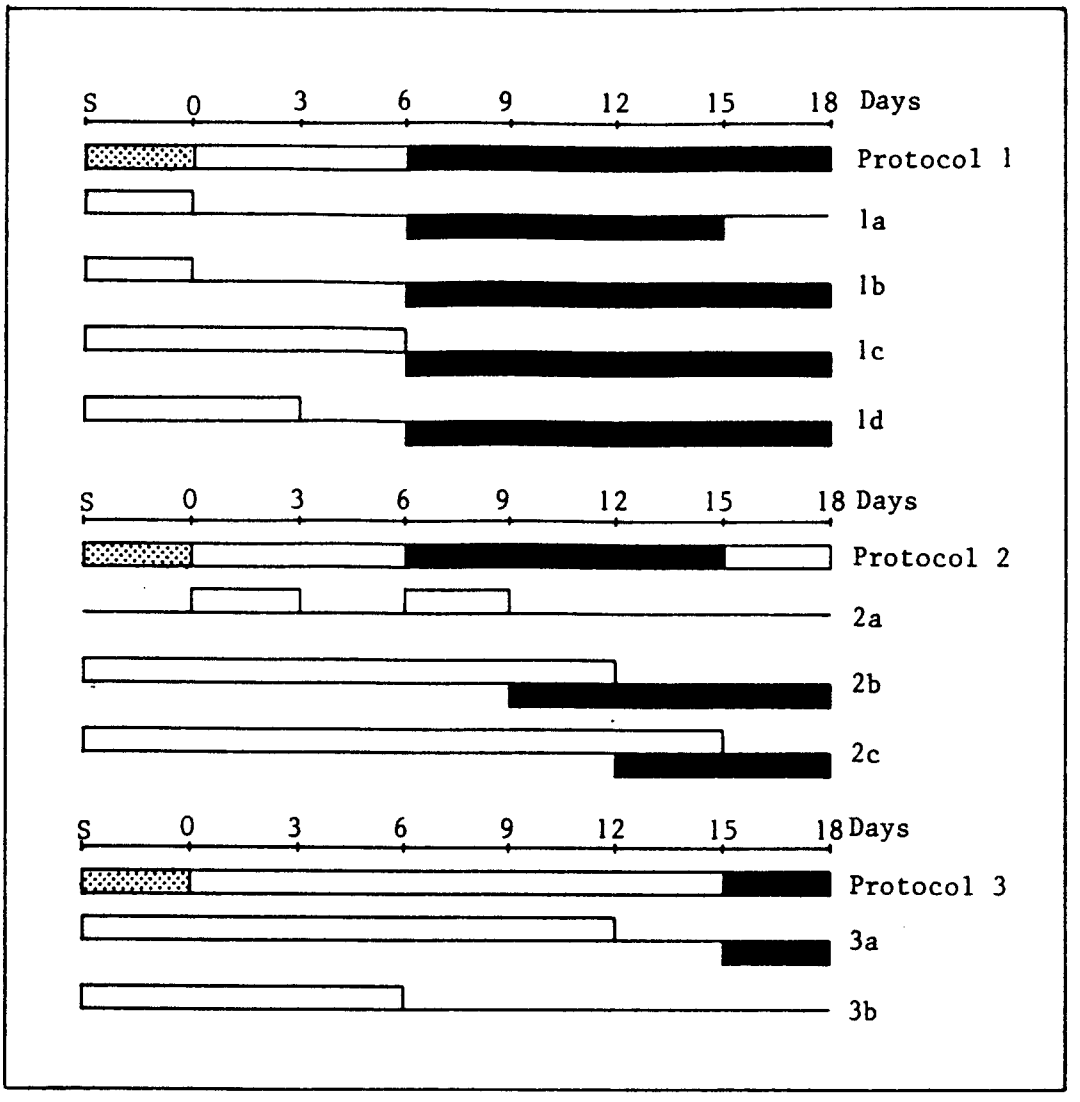


Figure 11. F_1 phenotypes from orange-eyed females after remating. See text for explanations.

strain 94.46 of D. sulfuriqaster (Tables 4 and 8). For clarity it is possible, though this may not strictly be correct, to treat these genotypes and phenotypes as if bright, dark and wild-type were dominant to orange. There are four phenotypic classes involved but discussion is greatly simplified if bright, dark and wild-type are lumped together and called wild-type. This way of naming is adopted below and the genotypes are arbitrarily given as o/o (homozygous, orange-eyed, 94.46), o/+ (heterozygous, wild-type) and +/+ (homozygous, wild-type, sulf-1).

In the four experimental protocols (1 - 4) single o/o females were first placed with o/o males for three days, long enough for homogamic matings to occur. They were then placed with +/+ males; the difference between protocols was in the timing and duration of confinement with the second males. In Figures 11 and 12 these different protocols are defined by the first horizontal bar in each set. For example, the first such bar in Figure 11 corresponding to Protocol 1 indicates that from the start (S) to the third day (D) females were confined with o/o males (stippled portion of bar); for the next six days (D - 6) they were without males altogether and then, on Day-6 (6 in the figure) +/+ males were placed with them for 12 days until Day-18.

The bars below the first bar in each set are the results. The result-bars or lines show the phenotypes of the progeny. For example, female 1a was transferred to a new vial every three days until Day-18. In each vial she oviposited; these seven vials were retained and as progeny emerged they were scored. The result line in the figure (1a, Fig. 11) shows that in the first vial she had o/o progeny, in the second and third vials she had no progeny, in the fourth to sixth vials she had o/+ progeny and in the last vial she left no progeny. All the females' progeny were orange-eyed (o/o) initially, this is represented by the white bars above the lines. If a heterogamic mating occurs (i.e. with a +/+ male) progeny would be heterozygous (o/+) for the marker, this is indicated by black bars below the lines (e.g., Fig. 11 : 1a, 1b, 1c, 1d).

In the first three test protocols flies were transferred every three days to fresh vials; old vials were retained for scoring. Protocol 4 was conducted over nine days and flies were transferred to new vials daily in order to examine in detail the period around Day-5.

The results show that D. sulfuriqaster females do remate. Of the 16 females tested 11 had progeny with two phenotypes while five females did not remate. However, it is not clear that remating occurred before sperm depletion. In some cases (1d and 3a, Fig. 11) it can be concluded that sperm had run out or degraded because the females stopped ovipositing (1d)

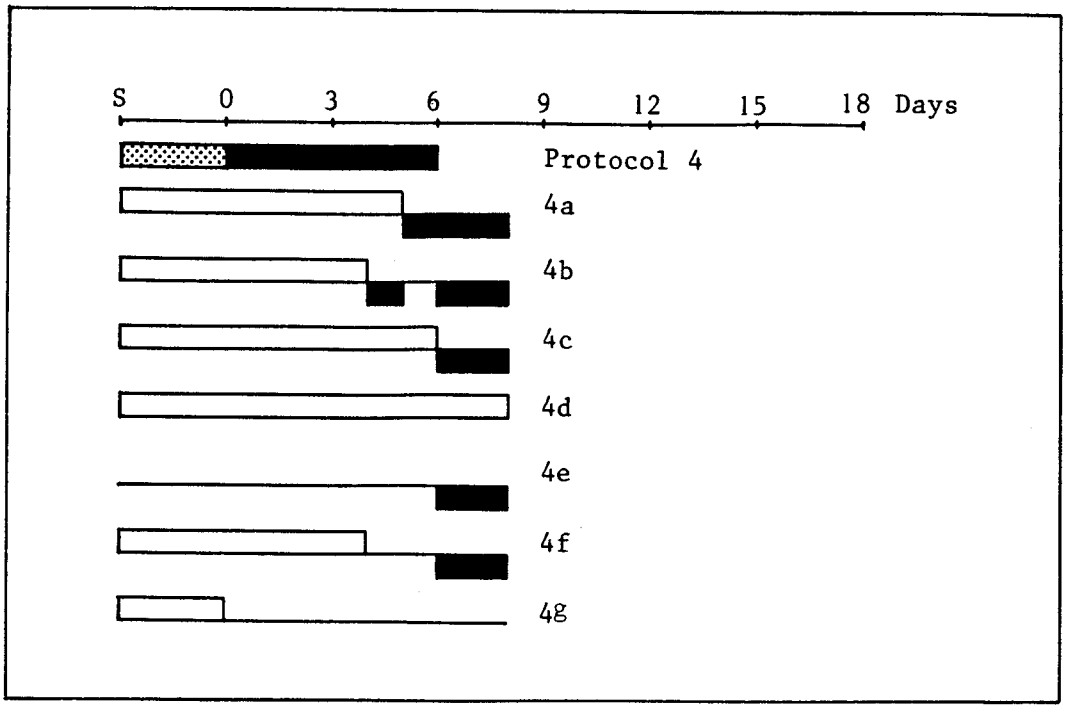


Figure 12. F_1 phenotypes from orange-eyed females (o/o) after remating. See text for explanations.

or began producing sterile eggs (3a) before they started producing o/+ progeny. Female 3a produced very high numbers of progeny, 157 in total, between the start and Day-9 but in the period between Day-9 and Day-12 only four progeny were produced and then between Day-12 and Day-15 she produced eggs which did not hatch. Whereas in other instances (1c, 2b and 2c, Fig. 11) the numbers of viable orange-eyed progeny (o/o) was still high when 'suddenly' they changed to wild-type (o/+) progeny. For example female 1c produced 56 orange-eyed progeny between Days-3 and -6 and then 71 wild-type progeny between Days-6 and -9 without a single example of mixed insemination. (The apparent overlap of progeny-types from females 2b and 2c is explained as an artifact of the scoring interval which was once every three days; progeny in the fourth experiment were scored at shorter, daily intervals.)

In order to force remating while sperm supplies are replete +/+ males were placed with females straight after the o/o males were removed (Day-0 in Protocol 4, Fig. 12). With this procedure no evidence of remating was found until four

days had elapsed (4b in Fig. 12). These data strongly suggest that while a female's store of sperm is full or near to full she does not remate or that the sperm of the second male is not utilized until depletion of the first's.

Sperm displacement has not been clearly demonstrated for D. sulfuriqaster. Certain females are able to utilize sperm from a first mate for at least 12 days, perhaps as many as 17 days (e.g., 2c) but others apparently exhaust their supply or are unable to utilize it after only two or three days (e.g., 1a, 1b). Progeny from a first mating were never observed after a second mating had occurred. The receptivity of D. melanoqaster females, for example, changes after they mate and two different but related factors are usually given as explanation: '(1) a "sperm effect" in which receptivity remains switched off so long as there are sperm in the storage organs of the female and/or (2) a 'copulation effect' in which the act of copulation itself has induced the lack of receptivity.' (Fowler 1973:329). Manning (1962) has suggested that a substance in the supporting medium of the sperm (i.e. the paragonial fluid) may inhibit receptivity. The problem is complex and remains unresolved (Fowler 1973, Jallon 1984). The phenomenon is also capricious - Fowler (1973) comments that 'it is a well known fact that the female rejection reactions displayed during the period of unreceptivity can be overcome by a particularly persistent male.'

It is assumed that females of nasuta and kepulauana or their hybrids utilize sperm in similar ways and show the same reluctance to remate while sperm storage is replete. This is a reasonable assumption since variation in the ability to remate is unknown within Drosophila species subgroups (Fowler 1973).

4.6 Copulation Duration and Sperm Transfer

In Drosophila the duration of copulation is fairly constant within species (Spieth 1952, Fowler 1973) but varies greatly between species ranging from about 30 s to 1.5 h across the genus (Fowler 1973). D. melanoqaster has been

extensively studied and although unmolested copulation lasts about 20 min it has been found to deviate to a lower limit of 10 min and an upper limit of 24 min (Manning 1962). Parsons (1967) concludes that the duration of copulation is largely under genetic control and Macbean and Parsons (1967) have suggested that the variation in copulation time may be proportional to the amount of sperm transferred (but see Fowler 1973 who disagrees). If D. nasuta and D. kepulauana have different copulation durations it will be possible to determine the direction of drift or selection of this trait in a hybrid population.

The time of the beginning and end of copulation is readily obtained from TRACE video analyses. Data are available for the three strains: nas-1, kep-1 and 09.F_{7-10} (i.e. F_{7-10} from population **09**) hybrids, which were filmed extensively for another investigation. Twenty 4-5 day-old virgin females and 20 4-7 day-old virgin males were placed together in an arena. Data from 37 12 h TRACE films were used. Only copulations preceded by the female acceptance posture were scored. The results are presented in Table 10.

The duration of copulation in nasuta and kepulauana is in the vicinity of 22 min. Excluding the kepulauana x nasuta value - which is based on only three variables - there is no significant difference between any of the times when the females are nasuta or kepulauana. The hybrid female copulation is extended. When hybrid males mate with nasuta or kepulauana females the duration of copulation is similar to the duration when these females mate with sibling males. Thus it may be concluded that the 09 hybrid females cause the prolongation.

It was possible to examine the sperm storage organs in females soon after the start of copulation (**Tsc**). Mating pairs were artificially separated at intervals to determine when sperm is transmitted. Twenty D. nasuta females were dissected. Two were dissected at **Tsc** + 2 min; no sperm was detected. Three were dissected at **Tsc** + 4 min; in one a small number of spermatozoa could be seen in the spermathecae but in the others no sperm could be observed. Five were dissected at **Tsc** + 5 min; one had large numbers of sperm in the spermathecae

and in the seminal receptacle; the remainder had none. Ten were dissected between **Tsc** + 5 min and **Tsc** + 10 min; all had sperm in both the spermathecae and the seminal receptacle. These 20 dissections indicate that sperm is transferred within 10 min of the start of copulation and only rarely within the first four minutes.

Table 10. The mean duration of copulation of D. nasuta (nas-1), D. kepulauana (kep-1) and F_{7-10} hybrids from population number 09; n: number of copulations scored.

female	male	n	mean	s.d.
nas-1	nas-1	34	22.76 min	3.24
nas-1	kep-1	69	22.21	3.78
nas-1	09	27	21.93	2.91
kep-1	nas-1	3	24.68	1.09
kep-1	kep-1	61	21.10	3.30
kep-1	09	16	24.15	4.29
09	nas-1	26	28.61	3.82
09	09	11	29.06	3.74

A forced copulation is not preceded by a female's acceptance posture. The duration of a forced copulation is significantly ($P<0.05$, Student's **t**-test) less than a 'normal' copulation. Forced copulations have an average duration of 2.6 min (n = 91, V = 4.97, s.d. = 2.2). These figures are derived from pooled scores for various combinations of male and female hybrids and non-hybrids. Forced copulations occur when flies are crowded together and when females are apparently unable to move away. The orientation and behaviour of flies is different during such copulations: males are almost at right angles to females; females move around (cf. stationary); females' wings are partially closed (cf. spread) and there is good evidence, given below, that sperm is not transferred.

A group of 20 females were dissected at the end of a 12 h period during which six 'normal copulations' and 14 'forced copulations' were observed. Five females had sperm in their storage organs and 14 did not. The 20th fly escaped. It was

not possible to identify the females which had shown acceptance and those that had not at the time of dissection. Therefore, although the evidence is circumstantial it does suggest that normal copulations result in sperm transfer and that copulation which is achieved without normal signalling does not. Another five females were isolated from males after forced copulations and allowed to oviposit. None of their eggs were fertilized. It can be concluded that forced copulations generally fail to result in sperm transfer.

The Specific-mate Recognition System (SMRS) is a male-female communication system which leads to fertilization (Paterson 1978, 1980, 1981, 1985). Copulation is essential for fertilization. In Drosophila the union will never occur by chance and a certain amount of 'cooperative' or 'coadapted' behaviour must precede it. Courtship increases the probability of copulation. In the previous section it was shown that D. sulfuriqaster females mate more than once. It was also found that remating never occurs before at least five days has elapsed. In section 4.3 it was shown that fertilized nasuta females were actively courted by males but that no matings resulted. In this section it has been shown that nasuta and kepulauana have similar copulation duration times and that sperm is transferred during the first 10 min. Hybrid females copulate for longer and it is suggested that this is caused by them and not by males. Those flies which attempt copulation before the normal sequence of signals and responses do not appear to achieve sperm transfer and fertilization.

4.7 Acoustic Signals

In many species of Drosophila wing vibration occurs before copulation (Spieth 1952, 1966, 1974). In the species of the D. nasuta subgroup the wing movements of males are elaborate and species-specific (Spieth 1969). It was demonstrated by Shorey (1962) that wing vibration by Drosophila produces sound. Recordings of the sounds made by different species led to the conclusion that they are species-

specific (Ewing and Bennet-Clark 1968; Ewing 1969, 1983; Bennet-Clark et al. 1980). Experiments on males with aberrant or no wings (vestigial mutants) demonstrated that they were generally unable to achieve copulation (Sturtevant 1915, Ewing 1964). Petit (1959) showed that amputation of the mechano-receptive part of the female antenna reduced female receptivity. She showed that the stimulus associated with wing-vibration was mechanical (i.e. acoustic) rather than chemical. Thus the theory that wing movement in Drosophila assists the dispersal of pheromones, as in certain other Diptera (Ewing 1977), now seems unlikely. Finally the most direct evidence that the sound production has adaptive significance and is part of the Specific-mate Recognition System was provided by a study of the electrophysiology of the antenna: Ewing (1978) found 'that Johnson's organ on the antenna of Drosophila is a sound receptor which is selectively tuned to the range of frequencies found in Drosophila songs' (Ewing 1983:283).

The sounds produced by over a hundred different Drosophila and Zaprionus species (Drosophilidae) have been described and reviewed by Ewing (1983). But only Leroy and Eucher (1981) have previously reported that sounds are associated with the wing movements of males and females of D. nasuta. Two types of male-sound were found which they called the 'émission longue' and 'émission brève'. (Details of the whole nasuta signalling system are given in section 4.10. In brief, males orient to the rear of females, they then circle around them to the other side extending and vibrating the wing closest to the female as they go. In contrast kepulauana males stay in front and rapidly lunge into and back away from females). The long emission of nasuta males is given when they are circling and the short emission when motionless. Females produce a sound both before and during copulation (Leroy and Eucher 1981).

What are the acoustic signals produced by D. kepulauana and other species in the nasuta subgroup? To record signals, pairs of flies were confined in a perspex cell fitted on the head of a microphone (Calrec CB7D unidirectional condenser microphone with a CC52 capsule; Fig. 13). A glass cover-slip

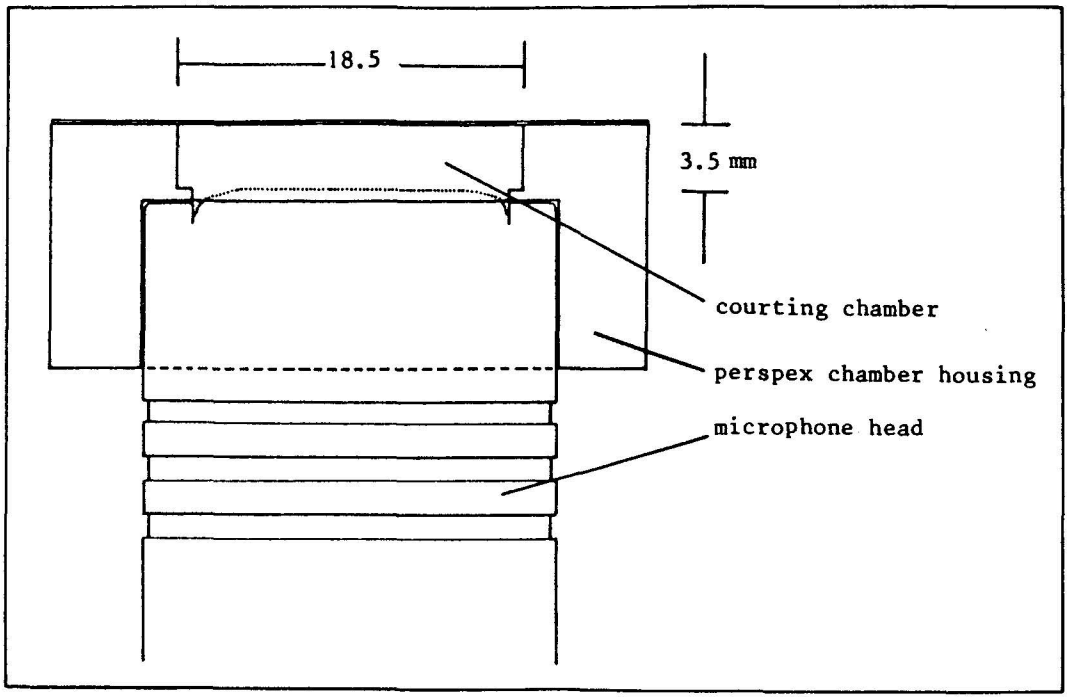


Figure 13. Plastic cell for recording acoustic signals. A lateral hole provided access for insertion by aspiration of flies; the cell fits snugly over a microphone head.

formed the top of the cell. A video camera (National WV-1500E; C, Fig. 8, p. 46) focused on the cell and recorded visual signals while acoustic signals were fed simultaneously (via line m, Fig. 8) to a video recorder ('Panasonic National 1/2" Time Lapse Video Recorder Model NV-8050'; VCR, Fig. 8). In the present study (cf. Crossley and McDonald 1980) the microphone was suspended between elastic bands inside a sound-proof box (SB, Fig. 8) as was done by Ewing and Bennet-Clark (1968) and the camera was mounted above it. The box was made of 5 cm thick panels (50 x 50 cm) of fibre-board; the base rested on a cushion of cotton wadding. The camera filmed through a small window. Three torch globes (2.4V 0.5A) powered by a Wild microscope transformer (T, Fig. 8) were mounted around the cell (G, Fig. 8) providing illumination without producing excessive heat or noise.

The cell volume (0.94 cm^3) was smaller than has previously been used for Drosophila studies (e.g., 5.73 cm^3,

Bennet-Clark and Ewing 1967; 1.13 cm^3, Shorey 1962; 2.42 cm^3 Crossley and McDonald 1980, Crossley 1986). However, the cross-sectional area was not greatly different and the height (3.5 mm vs 10 mm or 7 mm, loc.cit.) left ample clearance whether flies were mating or not. The smaller volume kept flies closer to the microphone head and produced clearer results. The cell diameter is approximately six body-lengths and males need only three to circle unimpeded.

Sonograms were prepared on a Kay model 7029A spectrum analyser (Kay Elemetrics Company, N.J., USA) within the frequency range 80-8000 Hz. Use of a wide-band filter (300 Hz) enhanced time resolution.

Six species were examined: D. nasuta, D. kepulauana, D. kohkoa, D. pallidifrons, D. sulfuriqaster and D. pulaua. Drosophila kepulauana was discovered to be almost silent in comparison with the very distinctive repertoire of nasuta. This is an important finding. Drosophila nasuta/kepulauana hybrids were examined. Five sonograms of hybrids and one of nasuta are reproduced in Fig. 14. The kepulauana signals were too faint to be resolved in sonograms.

Figure 14 shows that F_2 hybrids produce a range of nasuta-like signals but with short (Fig. 14.b) and very short durations (Fig. 14.c). In addition it is evident that certain hybrids make faint noises corresponding to rapid wing-alternation and lunges (Fig. 14.e-f), this affect is called kepulauana-like despite the fact that kepulauana s.st. is silent. It was found that the acoustic signal of F_1 males was like that of nasuta but with a duration about half the normal long-signal of nasuta (e.g., Fig. 14.c).

The frequency of pulses within a burst, unlike the duration of the burst itself, was found to be conservative. Note that the pulse-frequency of nasuta (Fig. 14.a) is reproduced almost exactly by two F_2 hybrid males (Figs 14.b and 14.c); the pulse-frequency in each of these first three sonograms is within the range 104+/-3 Hz. In the other hybrids (Figs 14.d-f) it is possible to detect similar low frequency acoustic signals but there are too few pulses and they are not well-resolved. However, on the basis of the time interval

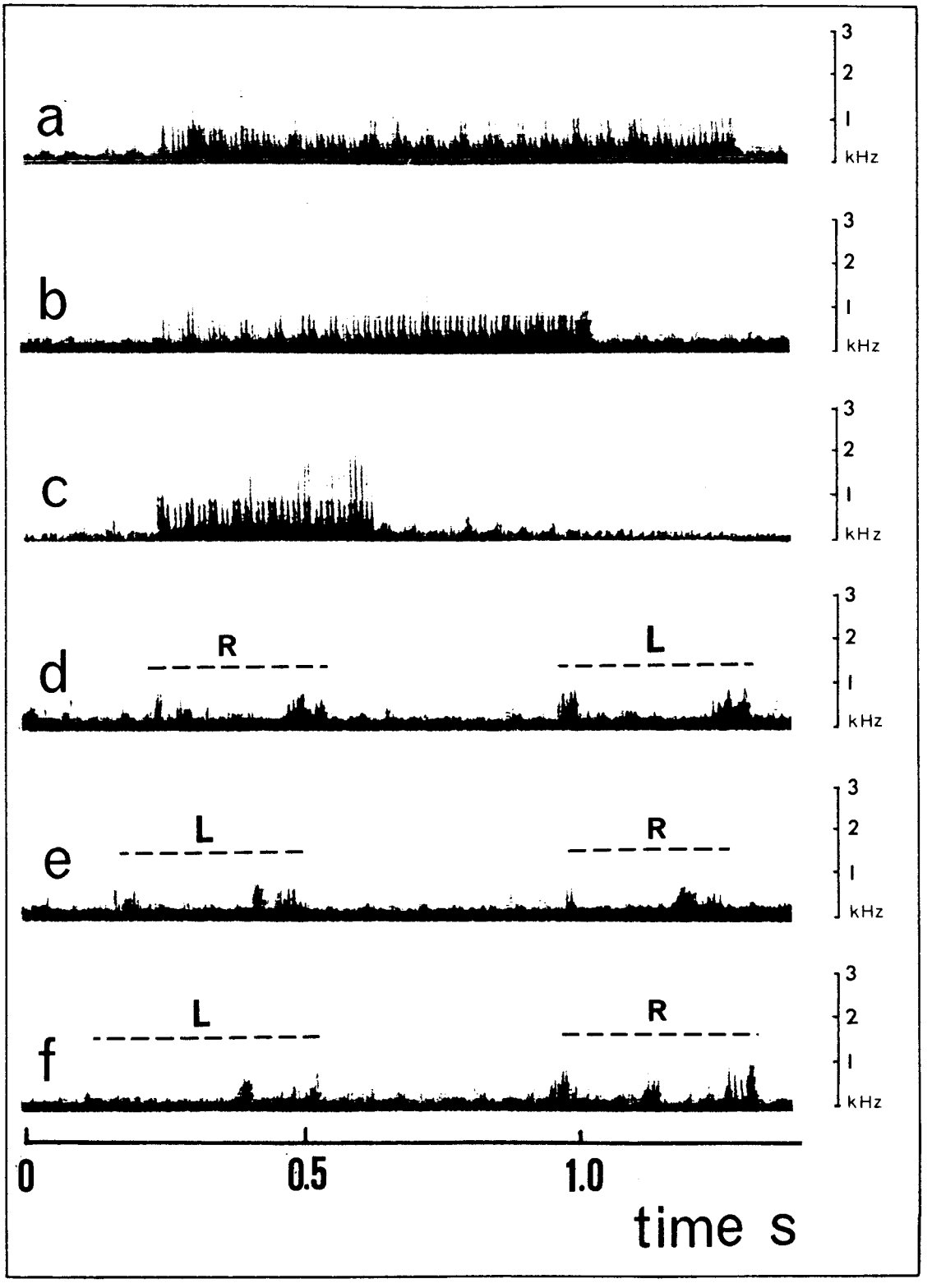


Figure 14. Sonograms of Drosophila nasuta and hybrids. Six sonograms: a, the 'long sound' of nasuta; b - f, nasuta-like sounds and sounds associated with kepulauana-like lunges made by different F_2 hybrid males; L, left wing; R, right wing.

between alternate wing bursts (c. 0.5 - 1.0 s) they can be interpreted as being similar to kepulauana (section 4.11).

Drosophila pallidifrons males produce a clearly audible acoustic signal when they extend and vibrate their wings. They produce high frequency short emissions and low frequency long emissions; females produce a sound while they are 'courted' and during copulation. There are qualitative and probably quantitative differences but the culture of pallidifrons died before recordings were made.

Like kepulauana the signals of kohkoa, sulfuriqaster and pulaua were feeble. By listening with headphones directly from line m (Fig. 8) it was possible to distinguish very faint pulses corresponding to the wing vibration during the 'twisting or side-to-side motion' (Spieth 1969) of sulfuriqaster. In contrast pulaua males held their wings still while they were outstretched 'at right-angles to the body' (Spieth 1969) and no acoustic signal whatsoever was audible.

Using a stroboflash Clark (1957) was able to determine that the 'wing-beat frequency' was c. 145 Hz for D. sulfuriqaster and that F_1 hybrids between strains with higher and lower frequencies were intermediate. Furthermore, he found that physiological differences which characterized the parental strains showed pronounced maternal bias in hybrids. He concluded that in contrast to the pattern of inheritence of physiological traits the inheritance of wing beat frequency is of the 'multifactorial type' (Clark 1957:220).

Leroy and Eucher (1981) describe three types of sound made by D. nasuta. The males produce two sounds. One only when they are circling in front of the female and one when they are stationary, usually behind the female but sometimes when they are in other positions. They describe the low frequency signal as 120Hz with a variable duration. They give 0.47 s +/- 0.10 as the average duration. In the present study longer times and slower frequencies have been more frequently recorded.

4.8 A Time Component of Courtship (ibi)

Data of an 'all or nothing' form poses interpretative difficulties in a study of the intermediate condition. However, the frequency of wing-extension per se can be readily scored for nasuta, kepulauana and hybrids and this yields data well-suited for an analysis of hybrids. The wings of kepulauana males alternate from left to right rapidly during courtship whereas nasuta alternates slowly. It is predicted that F_1 males will be intermediate. A method is given here for measuring and analysing the frequency of wing-extensions during courtship and some results are presented.

There is a periodicity about the way a female receives signals from a male; she does not receive a continuous signal rather it is as if she receives bursts of signals. In nasuta the wing extension is associated with a long acoustic signal and then a pause and in kepulauana with sudden left-right lunging motions with short pauses. The time between these bursts is called the **interburst-interval** or ibi. It is not the same parameter as the acoustic variable - the interpulse interval (ipi) - described by Kyriacou and Hall (1982). Crossley (1986) describes an interval between 'successive bursts of sound' which she calls the 'inter-burst interval'. For the present purposes the term: 'interburst-interval' does not imply the production of sound.

The time of the interval appears to be important. If, for example, a nasuta male has been signalling to a stationary female which then moves, it is characteristic to see him move faster thereby achieving a constant interburst-interval (ibi). This is despite the extra distance covered in order to achieve it. Thus the ibi is not necessarily a function of distance moved or to maximum speed of bodily movement. Rather it seems to be an interval which is timed neurologically, perhaps under the regulation of a gene like per in D. melanogaster (Kyriacou and Hall 1980).

TRACE films yield large samples of the ibi variable because wing-extensions are recorded against time (C, Table 2, p. 47), i.e. during 'tracking' the time is noted when a fly

extends a wing (C). Thus, time increments between C's in a bout of courtship are interburst-intervals. For example, in the hypothetical TRACE data-matrix in Table 2, male number 06 extends his wing three times producing one interburst-interval of 1 s and another of 2 s duration. A pause of 7 s or more between wing extensions has been arbitrarily treated as an upper limit and cut-off point, intervals longer than 6 s are not treated as ibi's.

Tables 11, 12 and 13 summarize the ibi results for kepulauana, nasuta and their hybrids. Part of these data are represented as a histogram in Fig. 15. The average ibi of D. kepulauana is 1.3 s (based on the signalling of five males); the ibi of D. nasuta is 2.8 s (34 males) and F_1 hybrids have an average ibi of 2.05 s (12 males).

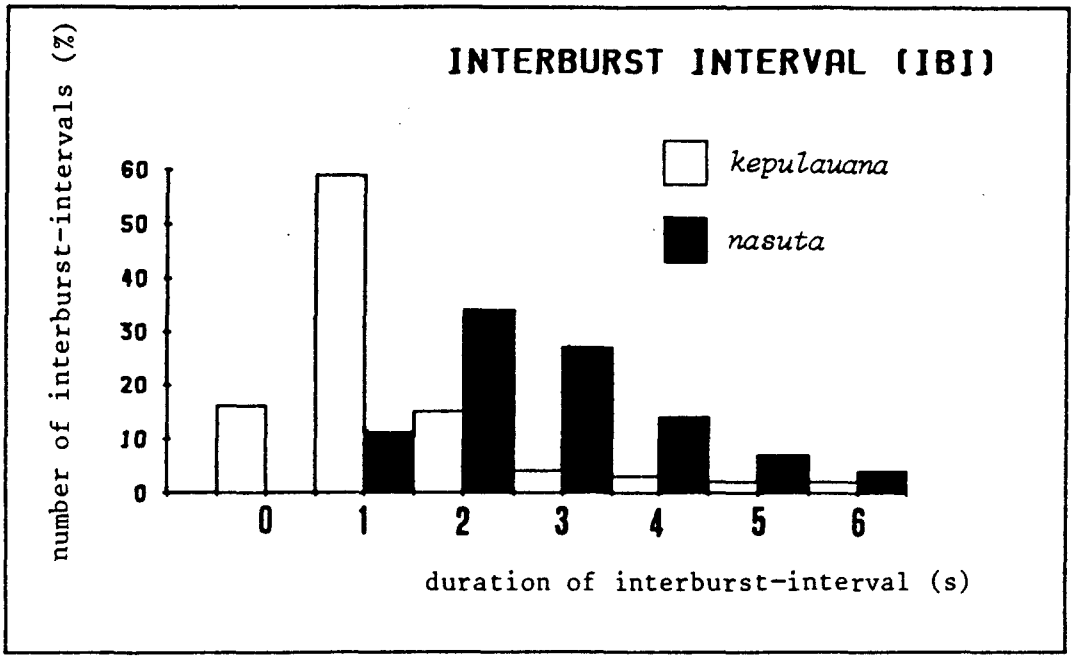


Figure 15. Frequency distribution of the interburst-interval of kepulauana and nasuta. Percentage distribution of samples given in Table 11.

The ibi distributions have been compared between males. But it has also been possible to look at the ibi's which occur just prior to when a female signals 'acceptance'. Is there a difference in the ibi's which a male delivers generally and

those which a female responds to? To what ibi do hybrid females respond?

Table 11. The distribution of the ibi variable among kepulauana, nasuta and hybrid males. F_{1-3} hybrids from population 26, F_{23} from 37. The distributions of the ibi variable marked 'fem' are associated with the female precopulation posture in three female groups. N, number of individuals examined; n, number of variables.

		ibi - classes (seconds)							
	N	**0-1**	**1-2**	**2-3**	**3-4**	**4-5**	**5-6**	**6-7**	**n**
kep	(5)	52	194	49	14	10	5	5	329
nas	(34)	0	99	437	287	145	74	37	1079
F1	(12)	3	208	232	72	44	13	11	583
F2	(8)	15	173	145	102	39	21	9	504
F3	(10)	0	23	77	30	6	8	2	146
F23	(14)	17	83	36	19	7	2	4	168
fem.kep	(6)	12	49	14	4	2	1	0	82
fem.nas	(15)	0	41	58	42	15	10	1	167
fem. F1	(5)	1	28	15	18	9	3	5	56

Table 12. The percentage frequency distribution of ibi variables. See Table 11 for abbreviations.

	ibi - classes (seconds)						
	0-1	**1-2**	**2-3**	**3-4**	**4-5**	**5-6**	**6-7**
kep	15.8	59.0	14.9	4.3	3.0	1.5	1.5
nas	0.0	9.2	40.5	26.6	13.4	6.9	3.4
F1	0.5	35.7	39.8	12.4	7.6	2.2	1.9
F2	3.0	34.3	28.8	20.2	7.7	4.2	1.8
F3	0.0	15.8	52.7	20.6	4.1	5.5	1.4
F23	10.1	49.4	21.4	11.3	4.2	1.2	2.4
fem.kep	14.6	59.8	17.1	4.9	2.4	1.2	0.0
fem.nas	0.0	24.6	34.7	25.2	9.0	6 0	0.6
fem. F1	1.3	35.4	19.0	22.8	11.4	3.8	6.3

The 'observed' distributions given in Table 11 are transformed into percentage 'frequency' distributions (Table 12). Two frequency distributions were tested for homogeneity by first using them to form a theoretical distribution here called the 'effective' distribution. The effective is an average of the two frequency distributions. In separate operations each of the frequency distributions can be tested against the effective distribution using chi-square. If the two frequency distributions are homogenous then they will not differ significantly from the effective distribution. In this way all distributions (Table 11) have been tested for homogeneity and the results are presented in Table 13.

Table 13. Significance of difference between the ibi frequency distributions of various male types. See Table 11 for abbreviations. The ibi samples (marked 'fem') which elicit the female acceptance posture are also tested for significance. (*, P<0.05; **, P<0.01; ***, P<0.001).

	kep	nas	F1	F2	F3	F23	fem kep	fem nas	fem F1
kep	n.s	***	***	***	***	n.s.	n.s.	***	***
nas		n.s.	***	***	n.s.	***	***	n.s.	***
F1			n.s.	n.s.	*	**	***	n.s.	*
F2				n.s.	**	*	***	n.s.	n.s.
F3					n.s.	***	***	n.s.	***
F23						n.s.	n.s.	***	**
fem.kep							n.s.	***	***
fem.nas								n.s.	*
fem. F1									n.s.

The kepulauana ibi is significantly different (P<0.001) from the nasuta ibi. The ibi of F_1 hybrids is also significantly different (P<0.001) from that of the parental species. In a single hybrid population over three generations the distribution of the interburst-interval changed significantly (P<0.05) in population 26 (Table 6). At the beginning the 26.F_1 males have an intermediate interburst-

interval. Indeed the mean interval 2.05 s (s.d. = 1.14) is an exact intermediate between the two parental means (1.3, s.d. = 1.13 and 2.8, s.d. = 1.23).

The distribution of the ibi variable among 26.F_1 and 26.F_2 males is significantly different ($P<0.001$). This could be explained as follows. All 26.F_1 males were heterozygous for parental alleles at all loci and were relatively homogenous but 26.F_2 males varied considerably following recombination in the F_1 and this would reflect as a greater spread in the distribution, the F_1 and F_2 would therefore have significantly different distributions. 26.F_3 males have an ibi which is not significantly different from nasuta. In other words it would not be possible to observe a difference between nasuta and 26.F_3 males in the periodicity of successive wing-extensions as they occur in front of females. Note how none of the F_3 hybrids had an ibi in the first class: 0 - 1 s (Table 12) unlike the former generation (F_2). However, in another hybrid population (number 37) after about 20 to 25 generations the males produce many short interburst-intervals like kepulauana ($P>0.05$) and unlike any other male type.

Females of kepulauana and nasuta responded to ibi's which had distributions not significantly different from the 'normal' ibi of their conspecific partners. This suggests that females and males are coadapted: males give signals to which their respective females are responsive. This result also demonstrates that females are responsive to the normal type of male ibi and that females do not mate when males have a faster or slower interburst-interval distribution. Whether the wing action is a mechanical or tactile stimulus or whether the air compression, sound or particulate disturbance resulting stimulates females has not been determined.

F_1 females respond to a different ($P<0.001$) distribution of interburst-intervals from that which is signalled by either kepulauana or nasuta males. Indeed the 26.F_1 females do not respond to the signal type of their own sibling males either i.e. the 26.F_1 males. They respond to an ibi which has a frequency profile not significantly different ($P>0.05$) from that of the heterogeneous F_2 male ibi (Table 13).

Various methods are available for 'estimating the minimum or effective number of genetic loci contributing to the difference in a metrical trait' (Lande 1981, Wright 1968). Estimates are based on phenotypic means and variances of a character in parental, F_1, F_2 and backcross populations. Three of the five important assumptions are, however, not met in the present study: (1) there is chromosomal recombination, (2) there is probably significant selection during the experiment and (3) all matings cannot be assumed to be at random in the population. No data are available for the interburst-interval of back-crossed flies.

The interburst-interval has been found to be a useful parameter in the study of the sexual signalling between the flies in the present work. The features of the distributions of the variable allow a test of homogeneity between different sets of data. The distributions yield interesting information about the coadaptedness between sexes and there is a very striking feature: the responsiveness of nasuta females to hybrid signals which will be discussed fully in section 6.4. This latter finding may be very important in understanding the nature of the realignment of the sexual communication system in advanced hybrid populations.

4.9 Mating Asymmetry of D. kepulauana and D. nasuta

During the course of these studies it was noticed that when nasuta and kepulauana are placed together both male types respond to at least some of the signals of both female types. The degree to which interspecific signalling results in interspecific mating is examined in this section. Does positive assortative mating occur? Is the female response to heterospecific courtship the same in nasuta and kepulauana? Is a female's response fixed or does it vary depending on experience?

(1) Does positive assortative mating occur? In two trials 30 flies (10 nasuta, 10 kepulauana and 10 hybrids) were placed together with sexes in equal proportion. Hybrids were 01.F_3 in

the first trial and about F_7 in the second trial. Hybrids were included because evidence of positive assortative mating in a 'noisy' environment is more compelling.

Table 14. The identity of copulating pairs when kepulauana, nasuta and hybrids are placed together. Code-numbers identify the individual females and males respectively. Remating males in boldface; Tl (see page v), min:s; P, the probability of copulation if mating is random.

code-numbers	female x male	Tl	P
09 x 20	hybrid F_3 x F_3 hybrid	1:47	0.33
05 x 30	nasuta x nasuta	3:41	0.14
12 x 25	kepulauana x kepulauana	5:03	0.15
07 x 26	hybrid F_3 x nasuta	5:34	0.13
02 x 18	nasuta x F_3 hybrid	5:43	0.10
10 x 22	hybrid F_3 x kepulauana	10:05	0.12
13 x 23	kepulauana x kepulauana	10:50	0.14
01 x 28	nasuta x nasuta	11:24	0.18
11 x 17	kepulauana x F_3 hybrid	13:15	0.15
15 x 24	kepulauana x kepulauana	14:59	0.11
14 x 21	kepulauana x kepulauana	18:47	0.04
08 x 16	hybrid F_3 x F_3 hybrid	24:20	0.25
40 x 57	hybrid F_7 x F_7 hybrid	6:11	0.11
39 x 54	hybrid F_7 x nasuta	10:13	0.10
37 x **60**	hybrid F_7 x nasuta	19:10	0.02
38 x **50**	hybrid F_7 x nasuta	21:13	0.07
36 x **48**	hybrid F_7 x F_7 hybrid	23:11	0.06
45 x 49	kepulauana x kepulauana	26:04	0.23
44 x 47	kepulauana x kepulauana	27:34	0.18
32 x 56	nasuta x nasuta	36:51	0.19
34 x **60**	nasuta x nasuta	45:34	0.12
31 x **50**	nasuta x nasuta	50:45	0.16
33 x **48**	nasuta x F_7 hybrid	56:25	0.17
43 x 47	kepulauana x kepulauana	67:19	0.31
35 x 52	nasuta x F_7 hybrid	99:34	0.11

The TRACE method was used. The time between introduction and copulation (Tl) was determined. The maximum Tl also indicates the length of the trial. The identity of the copulating pairs is given in Table 14; code-numbers (01 to 30 in the first trial and 31 to 60 in the second) of individual flies are given to indicate the flies which remated (boldface, Table 14).

The number and type of available flies is known when a mating occurs therefore it is possible to calculate the probability of a particular mating (P, Table 14). When flies mate with the least available type, P is low. Consistently low P values suggest that males are not mating with females at random.

A statistical test is used to compare the theoretical probability of mating with the confidence interval, the latter was computed from the proportions observed. The null hypothesis that mating between nasuta and kepulauana is random in mixed groups has been tested (Table 15). The outcome is that the degree of positive assortative mating in kepulauana is significant ($P<0.05$); that nasuta does not mate positively assortatively ($P<0.05$) and that interspecific crosses are significantly low ($P<0.05$) when the two species are placed together.

Table 15. Results of random mating test between nasuta (nas) and kepulauana (kep) in mixed groups (* $P<0.05$).

mating type	theoretic	obs.	conf.interval	Ho
kep x kep	1/4 (0.25)	7/12	0.316 - 0.808	*
nas x nas	1/4 (0.25)	5/12	0.192 - 0.684	n.s.
kep x nas	2/4 (0.50)	0/12	0.000 - 0.247	*

From the same data the amount and direction of courtship was determined using TRACE. The product of 'number of wing extensions' and 'ibi' is a measure of the 'time spent courting' (T.sex) for each male. Thus, it has been estimated in the first trial that nasuta males spent 45% of their sexually active time courting conspecific females, 18% of

their time with hybrid females and 37% of their time courting kepulauana females. Whereas kepulauana males spent 89% of their sexually active time courting conspecifics and only 7% and 4% of their time with hybrids and nasuta females, respectively. It should be noted that here (cf. Chapter 5) percentages are based only on long sexual sequences. From these data it can be suggested that the reason why kepulauana mates assortatively is that kepulauana males spend most of their sexually active time courting kepulauana females and that signals which they receive from nasuta or hybrid females do not prompt them to begin or to maintain courtship with them. I treat positive assortative courtship and positive assortative mating as distinct. Furthermore it is apparent that nasuta males respond, under confined conditions, to the signals received from female kepulauana and female hybrids but not to the same extent as to signals from conspecific females.

(2) Is the female response to heterospecific courtship the same in nasuta and kepulauana? This question is not completely answered by the above data because the amount of time kepulauana males spend signalling to nasuta females is insufficient to judge whether the female response is similar in both species.

An experimental procedure in which females are repeatedly courted by only one type of male is appropriate here. It is often reported that depriving females their conspecific males increases the likelihood of heterogamy or hybridization (Spieth 1966, Mayr and Dobzhansky 1945, Mayr 1963). If the result of such a test - the so-called 'no-choice' test - is negative and little or no hybridization has occurred, then one has a high degree of confidence that under most other situations mating will not occur. So negative results from 'no-choice' experiments are therefore the most telling. Also it is probable that when tests are done in relatively open conditions only closely coadapted signal-response systems will work, more especially those systems which evolved in those particular 'open conditions'. The following method, which will be used extensively later to test advanced hybrids, is a 'no-choice' test conducted under relatively open conditions: a

much larger 'arena' than the observation arena used with TRACE.

The following results are a summary of the controls for a large series of 'no-choice, open-condition' experiments involving advanced hybrid males and nasuta and kepulauana females. The method will be given in Chapter 6 in which the most extensive use will be made of it. In brief, male and female flies were placed together in a large (11 l) plastic bucket with a squashed banana in the bottom and gauze over the top. When a mating occurred the pair were removed. Each control trial involved 50 virgin nasuta or kepulauana females and 60 nasuta or kepulauana females. The data from these inter- and intraspecific combinations form the control scores in the extensive studies of advanced hybrids (Chapter 6) but are also useful here.

The third and fourth data columns of Tables 1 and 2 in Appendix B represent the number of nasuta or kepulauana females mated (out of 50) during 320 min observation periods. Table 16 is a summary of that data, it gives the average percentage of females mated.

Table 16. The percentage of females mated when 50 are available under 'no-choice' open conditions.

		nasuta male	**kepulauana male**
female	**nasuta**	92.6	32.0
female	**kepulauana**	4.6	49.6

The asymmetry of results in Table 16 is important. The nasuta x nasuta result is very high and indicates that the SMRS of this species functions well under these particular open conditions. The kepulauana x kepulauana result is surprisingly low by comparison; only 49.6% of available females mate. The kepulauana strain used (kep-1, Table 3) has been laboratory bound since 1968 (c. 400-500 generations). It is probable that the SMRS of kepulauana is coadapted within the context of a particular array of environmental signals not present in a bucket. Obviously male and female kepulauana in

the vast tropical jungles of Borneo respond to that environment (their 'usual habitat' sensu Paterson loc.cit.) in such a way that they come into close proximity with members of the opposite sex. The question is whether the kep-1 strain has lost elements of behaviour which are important for achieving fertilization in large spaces where female density is low. The appropriate test (kep-1 vs a freshly collected kepulauana strain) was not possible - no 'fresh' kepulauana strains are available.

It is not appropriate to compare the rate of copulation between populations. One should only expect to find the 'most efficient' rate of mating that has ever evolved within an interbreeding population in a particular habitat, one which results in the maximum mean reproductive performance of individuals regardless of the long term population survival (Williams 1966:21, 28, 31).

Females do not respond to heterospecific courtship in the same way. When females receive only one type of signal they respond - positively - significantly more often to their conspecific males than to others (Table 15). Under open conditions 92.6% of nasuta females achieve fertilization when all the males are nasuta, when they are all kepulauana only 32.0% of the females become fertilized. When only nasuta males are present few (4.6%) kepulauana females mate with them but it was noted that the copulations which resulted were of short duration and intromission was apparently not complete. Therefore it is probable that the kepulauana females had not given the final precopulatory signal.

Unlike the 'choice' experiments discussed above the results here show that when nasuta females have 'no-choice' they will mate with kepulauana males. This partially answers the third question:

(3) Is a female's response fixed or does it vary depending on experience? Female kepulauana rarely mate with nasuta. Females of nasuta, on the other hand, will mate with kepulauana males especially under 'no-choice' situations.

It has been suggested that the direction of evolution of

two species may be inferred from evidence of asymmetric female response? Two hypotheses have been proposed: Kaneshiro's (1976) hypothesis would predict that kepulauana is the ancestral species and nasuta the derived species; whereas Watanabe and Kawanishi's (1979) hypothesis would predict the reverse i.e. kepulauana is the derived species and nasuta is the ancestral.

Kaneshiro's hypothesis (1976) is based on the idea that behavioural elements may be changed (lost) during the genetic revolution accompanying founder events. In his words (1976:743-744) 'a founder individual (single fertilized female) represents only a portion of the total gene pool of the ancestral population. The courtship pattern of the derived species therefore has elements in common with its ancestral population; but, on the other hand, a few elements of the total courtship pattern of the ancestral population are changed ("lost") by the "genetic revolution" which accompanies the founder event in the derived population. In this way females of derived species may recognize and accept the courtship overtures of males of ancestral species since these males contain all the courtship elements present in conspecific males. However, females of ancestral species show strong discrimination against males of derived species since these males contain only a portion of the total courtship pattern of conspecific males.'

Watanabe and Kawanishi (1979:907) have proposed a model which rests squarely on assumptions made by the Fisher-Dobzhansky model of speciation. They write: 'A newly evolved species would have a small population size and would be exposed to the danger of mixing with the original species. If the newly evolved females mate with the original males depletion of the new population results. But if a male of the new population mates with a female of the original population he may mate again with a female of the new population; therefore, the new population is not adversely affected. Genes introduced from the new population into the old will soon be eliminated from the original species by normalizing selection. Thus, the failure of matings between the males of ancestral

species and the females of the new species population would be the first step in developing reproductive isolation'.

Some weaknesses in both theories have been discussed by Lambert (1984a). The Kaneshiro hypothesis does not address the possibility that Specific-mate Recognition Systems are more realistically portrayed as having variable responses to particular signals i.e. SMRS's are more often 'non-linear' (Lambert 1984a). The hypothesis would, therefore, be more applicable to situations which involve the loss of terminal elements i.e. components at the end of behavioural sequences. The loss of 'middle' elements may, in some cases, be by-passed and of limited consequence. The applicability of Kaneshiro's hypothesis would therefore be limited to behaviour like that of the Ten-spined Sticklebacks for example (Morris 1970).

Lambert (1984a) and Kaneshiro and Kurihara (1981) note some serious errors in the basic assumptions made in the Watanabe and Kawanishi model. Lambert (1984a) demonstrated that it is teleological with reproductive isolation and speciation as goals. For example, note the use of the words: 'the danger of mixing' and 'The most important change for the creation of a new species is the failure of matings between the males of ancestral species and the females of the new species' (Watanabe and Kawanishi 1979:907). The whole notion of speciation by reinforcement and the evolution of mechanisms for reproductive isolation has been severely criticized by Paterson (1980, 1981, 1985). Furthermore, Kaneshiro and Kurihara (1981) argue that the evidence of corroboration used by Watanabe and Kawanishi (1979) is arbitrary and open to opposite interpretations with respect to the 'direction' of speciation in phylogenies.

Lambert (1984a) concludes that many speciation events will result in no change in the total number of elements in the new SMRS. Kaneshiro (1976:743) may not disagree with this for he actually uses the word 'changed' in preference to 'loss' but, on the other hand, he views derived courtship as 'only a portion' of ancestral courtship. Lambert (1984a:153-154) speculates that 'Divergent non-overlapping SMRS's could ... result from quantitative changes in any signal/receiver

characteristic e.g., changes in frequency of wing beat ... Asymmetrical evolution could result from differences in male frequency changes in such a case.'

The results presented in this and previous sections suggest that changes not only in male signalling but also in female receptivity could lead to asymmetric mating. It has been shown that nasuta females respond to a more variable form of male signal. For example, note that they respond to a wider range of the ibi variable (Table 13, section 4.8) and they mate with kepulauana males (in 'no-choice' experiments). Note also that nasuta males respond to a more variable form of female signal. For example, nasuta males spend approximately 37% of their sexually active time courting kepulauana females whereas kepulauana males spend only about 4% of their time courting nasuta females; this suggests that nasuta males are stimulated by a more variable type of female signal. Thus it could be concluded that kepulauana is tightly coadapted between the sexes and that nasuta, by comparison, is less so. This evidently leads to asymmetric mating behaviour. It is more parsimonious to view the asymmetry as an effect of a differential 'range of receptivity and responsiveness' rather than concluding that it is the consequence of the evolution of an ad hoc isolating mechanism in kepulauana.

Two final points need to be mentioned. Firstly, the kepulauana strain used in these experiments has had a very long laboratory life (nearly 20 years) and as a result it is probably significantly more homogeneous in phenotype or behaviour than the nasuta strains used. This is so despite the fact that the nas-1 strain is an iso-female culture. Furthermore it is probable that the sexual signals which exist among kep-1 flies are those which are best adapted to confined conditions of high fly-density such as occurs in culturing bottles where they have lived for hundreds of generations. This may explain why so few matings occur in open conditions compared to the nasuta strain which has been lab-bound for only two years. The second point concerns an interpretation of the visible differences in the SMRS's of the two species. It is possible to argue that a single change in a time component

of courtship accounts for all visible and acoustic differences. In essence if females change in their receptivity so that they only respond to rapid anterior wing signalling then males will not have time to circle and must remain anterior to females during bouts, moving to the female's posterior end only at the end of bouts. This interpretation favours Kaneshiro's line of reasoning since a significant change in the SMRS results from a single elemental change in the behavioural repertoire (Kaneshiro 1980:442); but we are still in the dark about which species is ancestral.

4.10 The SMRS of Drosophila nasuta

The SMRS is adapted to the habitat in which it evolved: the 'usual habitat' (Paterson 1985). The usual habitat of kepulauana is wet rainforest in Borneo (section 2.6). In contrast nasuta is adapted to more wide-ranging conditions (section 2.5). During the present study it has been observed mating in damp river-gallery forest and in semi-domesticated conditions in Swaziland and Madagascar. In the usual habitat the Fertilization System orients flies to places where their chance of encountering mating partners is high after which the SMRS may lead to syngamy.

The elements of sexual behaviour may vary in importance. The presence of one pheromonal odour may be mandatory while a male action like bobbing during circling may be present or absent. A sexual recognition system always involves signals and responses but it may not always be true that they unfold in a strictly linear sequence (Lambert 1984a). It seems more likely that various channels of communication are used simultaneously. This makes sexual behaviour difficult to reduce to component parts or elements and difficult to define as a sequence like: signal 1 then response 1 then signal 2 etc. The fertilization system comprises 'tuned receivers' to 'specific signals' but, of course, these may not be in channels to which we are sensitive.

It is convenient to study the visual channels of

communication of nasuta and kepulauana because the behavioural repertoires are rich with peculiar and repeated actions which may be recorded on video for detailed examination. However, it is likely that various acoustic, tactile, odorous and pheromonal signals also play important roles in the SMRS as they do in other Drosophila species (Ewing 1983).

Drosophila nasuta flies have been filmed. Detailed descriptions of the movements and the characteristic positions of the male with respect to the female at different times during 'courtship' are given below.

The first detectable signal to which males respond is movement. Tompkins et al. (1982) have shown that moving D. melanogaster females are more stimulating than resting ones. The same is true among groups of nasuta and kepulauana. When a fly moves near another, whether the other is moving or not, each extend their forelegs. Tarsi and tibia come into contact in this way. This is called 'legging'. Through legging males appear to obtain information about the sex of the other fly involved. Clark (1957:217) notes that 'contact with the fore legs [in D. sulfurigaster] leads to recognition of another individual as being of the same or opposite sex.'

'Legging' is distinct from 'tapping', a term Spieth (1969) uses to describe the action peculiar to males when they are close to and facing the posterior part of the abdomen of females; 'tapping' results in contact between the fore-tarsi of males and the body of females. Whereas legging results only in tarsi-to-tarsi contact. Another leg action - 'pawing' - is characteristic of males of D. sulfurigaster, D. pallidifrons and D. kohkoa but not of D. pulaua (Spieth 1969), D. nasuta nor D. kepulauana. Pawing is when a male taps the substratum with one and then the other of his fore tarsi while standing at a short distance (0.5 to 1.0 cm) diagonally in front of females (Spieth 1969). Typically males of the above species which 'paw' or 'tap' do so after a bout of wing-extensions.

Since no detectable sound is produced during legging the important stimulus (if one exists) is probably either tactile, a cuticular pheromone, or perhaps an air-borne pheromone active over very short distances. Visual signals seem much

less likely to be important at this stage. Drosophila nasuta subgroup females are morphologically very similar and apparently do nothing when being 'legged' by a male and yet termination results if certain males 'leg' certain females. It is very likely, however, that a nasuta male uses visual stimuli in order to orient to the posterior end of the female initially and to circle and track her subsequently; trial and error is not involved in this orientation. No investigation of pheromones was carried out although the results in section 4.3 provide some evidence that virgin females excite males without signalling to them in visual or tactile channels.

Males move up to and orient towards females at random; this suggests that a later step in the courting sequence fails to elicit a positive response in heterotypic interactions. It can be implied that something other than a visual or auditory cue is attractive initially but that it is not greatly different in females of nasuta and kepulauana. Antony and Jallon (1982) have shown that cuticles of female D. melanogaster have potential aphrodisiac substances and it is known that cuticular hydrocarbons from many insects are behaviourally active (Howard and Blomquist 1982). It has also been demonstrated that female compounds induce male precopulatory behaviour in melanogaster (Venard and Jallon 1980) and that the hydrocarbons involved are chemically similar among closely related species (Cobb pers.comm.).

If a nasuta male is stimulated positively by legging he will attempt to move to a position diagonally at the rear of the female (Figs 18.1 and 18.5). Spieth (1969) records an angle of 30° from the female mid-line, the same angle was found in the present study and by Lambert (1982). If an obstacle is present the male will edge his way between it and the female so that the characteristic 'starting position' is achieved. Females often but not always begin producing acoustic signals at this time using their wings. Females which appear to be agitated and are moving away from courting males produce similar sounds so they are difficult signals to interpret.

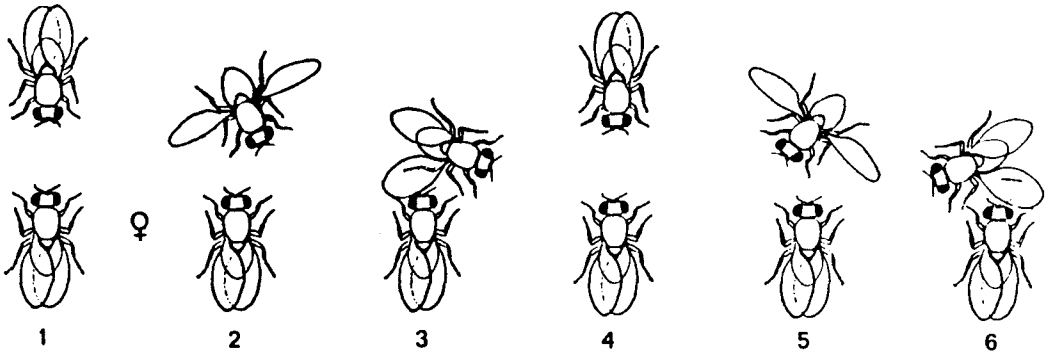


Figure 16. *Drosophila kepulauana* courtship

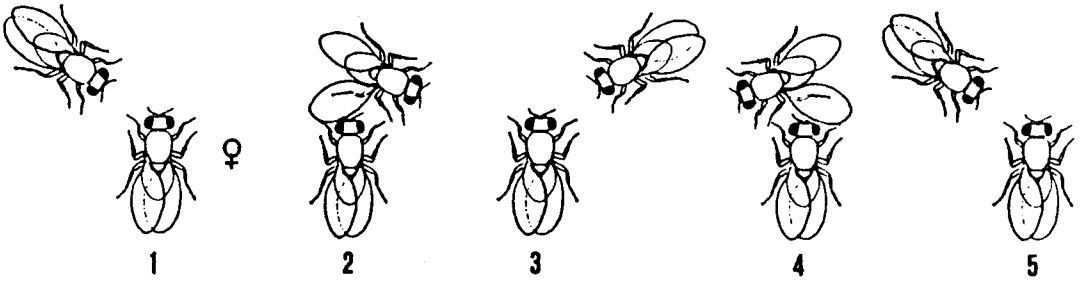


Figure 17. F_1 hybrid courtship

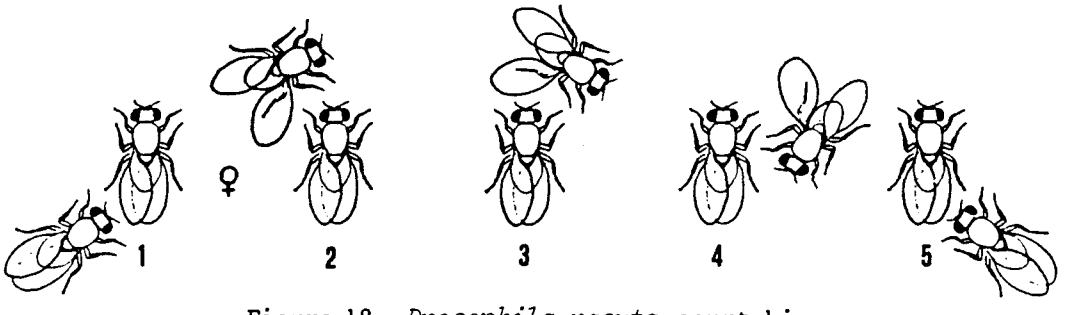


Figure 18. *Drosophila nasuta* courtship

Figures 16 - 18. The courtship of Drosophila kepulauana (16), F_1 hybrid (from kep male x nas) (17) and D. nasuta (18). 'Starting Positions' 16.1, 17.1 and 18.1 are consistently different. The time that elapses between 16.3 and 16.6 or 17.2 and 17.4 is an interburst-interval or ibi (see section 4.8).

The 'starting cue' which causes the male to begin to move from the 'starting position' is not clearly apparent. Some males wait motionless for long periods before beginning, others start immediately, while a third category follow females which move away from them. The time which elapses between when 'orientation begins' and when the first signalling or wing extending occurs is highly variable. For example, a nasuta male which was tracked during a TRACE trial paused for short periods (3, 1, 6, 3, 9, 6 s) and for long periods (23, 22 s) after orienting towards different females before commencing circling. The delay seems to depend on whether he has space to circle i.e. on the position of the female with respect to other flies. It therefore seems that 'beginning' is not prompted by an internal 'clock' in males. Nor does the cue seem to be a visible or acoustic signal from the female since, as far as can be detected, no unique action or noise precedes the start of circling. However, the apparatus used is not designed to sense small fluctuations in the 'state' (aromatic, physical, chemical etc.) of females. Fluctuations to which - it seems very probable - males are extremely sensitive.

Once 'started' a male moves in a path which Spieth (1969:260) describes as 'horseshoe shaped' around the front of the female (Fig. 18.1 through 18.3 to 18.5, p. 104) to a position exactly on the opposite side of where he started. Importantly the male does not move with a 'sideways crab-like' motion during circling like other species in the subgroup. Thus as he moves past the anterior of the female he is at right-angles to her longitudinal axis (Figs 18.2 and 18.3). The male pauses once he reaches a mirror-image position (Fig. 18.5) on the other side of the female before circling back again to the 'starting position' (Fig. 18.1).

During the anterior 'circling' movement the male's wing closest to the female is extended to an angle of 30 - 40° (in agreement with Spieth 1969:260), lowered slightly and vibrated. When males move between the figured positions 18.2 and 18.4 a distinct sound - the 'émission longue' (Leroy and Eucher 1981) - is produced by the wing (Fig. 14.a). The

duration of the sound is about one second but shorter sounds half this time have been observed in nasuta (e.g., 0.47 s, Leroy and Eucher 1981). The duration of sound produced by a single male varies under different circumstances, for example, if he is interrupted or if the female is cornered; these extraneous factors lead to signal brevity but nothing seems to provoke signal prolongation. (Signals extending beyond 1.4 s have not been recorded; indeed none would be expected given that the average interval between the 'middle' of one wing-extension and sound burst and another is 2.80 s (section 4.8) and for more than half this time the males wings are closed.)

Another sound is made by D. nasuta but copulation may be reached without it having been produced. It is important here only in as much as indicating that the males are capable of producing a signal with a different frequency although normally they do not. The short burst signal is made when the male is stationary and at the starting position; it has been heard only after males have repeatedly courted. The short burst signal has a much higher frequency (c. 425 Hz) and it also has a short duration - about 0.02 s (Leroy and Eucher 1981).

When males are in a starting position they are closest to the genitalia of the female and it is common to see a male tapping the female vigorously while in this position. However females were never seen to accept males that were in a 'starting position' tapping.

Females move short distances (c. 5 mm) just prior to copulation. The acceptance posture is similar in all species examined: the female spins around opening wings and depressing abdomen; the male rushes forward as a continuation of his circling motion and mounts. It is only during the long acoustic signal that females have been seen to accept males.

During the circling phase of the nasuta courtship males bob up and down. The bobbing action would effectively cause the frontal pollinosity (a 'dusty' white covering which has a high reflectivity at certain angles) to flash on and off because when viewed from certain angles (e.g., diagonally at 45°, Spieth 1969) the sheen is brilliant and from other

perspectives it is invisible. This is reminiscent of the flashing-effect observed in certain species of Zaprionus (Drosophilidae) which shake from side to side during courtship causing thoracic bands of pollinosity to appear to flash stroboscopically in the mid-line (Bennet-Clark et al. 1980). Although it would be interesting to experiment with apollinose males or blind females this has not been done. If the female perception of a flashing pollinose frons was an essential element of the SMRS then obviously fertilization would not occur under absolute darkness. However, fertility **is** eventually achieved under dark conditions (Wright 1977b). It can therefore be said that the visibility of frontal pollinosity is not, in itself, an vital part of the male's sexual repertoire. So although it may enhance the probability of fertilization it is not essential.

On some occasions males stimulated by the acceptance posture of the courted female were seen to rush forward but were 'off-line' stopping beside the female instead of on top of her. A long pause followed and if the female was still nearby courtship began again. In such cases females would almost immediately accept the male. This indicates a simple signal-response chain of events viz. signal - 'acceptance posture', response - 'move forward quickly'. Once a female was observed to display the acceptance posture while a male courted another female nearby; presumably the 'émission longue' acoustic signal was sufficient to cause the female to display the final pre-copulation signal.

4.11 The SMRS of Drosophila kepulauana

Drosophila kepulauana males produce no detectable sounds during courtship; a contrast to the prominent acoustic signal of nasuta. Males of kepulauana and nasuta start in different positions. For ease of description imagine the face of a clock with a female in the middle facing 12 o'clock, nasuta males start at 4:30 or 7:30 (Fig. 18.1 or 18.5) but kepulauana males start at 12:00 (Figs 16.1 and 16.4). A kepulauana male which

by chance approaches from the rear will extend legs towards the hind parts of the female, the female may extend her hind legs in response, the male may then move to a position in front of her. Whereas a male which approaches from the front usually begins a frontal display and then moves to the rear secondarily. It should be noted that 'legging' is something that is always associated with these flies when they move among other flies. Since kepulauana males that orient firstly to the front have not been seen to then move immediately to a position behind a female, it is suggested here that the 'starting position' for D. kepulauana males is 3 - 4 mm directly in front of the female (Fig. 16.1 cf. Spieth 1969, his Fig. 3).

From the frontal position males lunge forward and sideways (Figs 16.2 then 16.3) to a right-angular position in front of females (Fig. 16.3). This position and the position of a nasuta male as he moves past the front of a female are similar (Figs 16.3 cf. 18.3).

The lunging movement is rapid but it is possible to detect that both wings spread at the start (Fig. 16.2), only the one closest to the female remains extended when the male 'strikes' the female. Spieth (1969:261) describes the movement as 'lunging-wing-throwing movement'. The costa strikes either the legs or the face of the female. The male immediately moves back and then lunges forward again but with his other side (Figs 16.4 to 16.6). If uninterrupted the series of lunges eventually stops and the male moves behind the female; he may then return to the front and recommences lunging. A series of such sexual signals punctuated by a pause of greater than 7 s is a bout. There are an average of 7.3 (n = 83, s.d. = 3.7) lunges per bout. The ibi is 1.30 s (n = 329; see section 4.8). Therefore the average kepulauana bout of frontal stimulation lasts for 9.5 s.

In comparison, bouts of nasuta courtship consist, on average, of 3.75 frontal displays which are at 2.8 s intervals; nasuta bouts have an average duration of 10.5 s. The elements of courtship are strikingly different in nasuta and kepulauana but the duration of 'bouts' are approximately

equal. Sexual signalling with wings is not continuous it is punctuated every c. 10 s by pauses of greater than 7 s.

At the end of a bout of 'lunging' a kepulauana male will circle the female 'legging' her continuously as he goes. He does not lick when in a position behind the female as is typical in melanogaster. Females extend one or two legs towards the male and at the same time the male extends his fore- or mid-leg towards her as he circles. Legs are always in contact during circling.

Just prior to copulation females move slightly - a few millimeters - then as the male lunges during a bout she spins around with open wings, her exposed and depressed abdomen confronts the male. The male moves forward and mounts. Female acceptance has only been observed while a male is in an anterior position 'lunging', it does not occur when a male is in a posterior position or when the male is moving from behind to in front. Like nasuta, females accept males only while they are being stimulated anteriorly.

4.12 Summary

The objective of the overall work is to study the fate of hybrid sexual behaviour. In this chapter I have presented a number of studies on the reproductive biology of the two species (and some others in the same subgroup) which will form the gene pools for the hybrid populations. Understanding their reproductive biology is an essential preliminary before experimentation can begin on the outcome of introgression and natural selection.

In section 4.2 the time for an adult nasuta or kepulauana female to produce adult progeny was measured. The minimum generation-time (i.e. eclosion to eclosion) of nasuta was 13 days and for kepulauana was 14 days. The minimum time from egg to adult is between 10 and 11 days. Since the procedure for handling cultures (including hybrid populations) involves transfer of a random 'group' of adults at 15 to 20 day intervals, neither generation-time is at a disadvantage.

Eleven days is sufficient time for mature adult nasuta and kepulauana flies to complete a life cycle in a fresh bottle. The number of progeny produced by the two species was also measured under lab conditions and an important difference was found in this parameter: nasuta is more fecund. In terms of simple deterministic theory a hypothetical culture of two non-interbreeding species with the above differences in fecundity will, after about 10 generations, consist entirely of the species with a fecundity similar to nasuta.

The sexual activity of nasuta varies markedly over a 24 hour period and alters slightly depending on the condition of females present. In section 4.3 data are presented which show that male sexual activity peaks just after simulated dawn; all copulations occur during this period. When there is no light males are stimulated by virgin but not by fertilized females. Fertilized females stimulate males to court at artificial dawn but they do not mate with them.

Males of kepulauana reach sexual maturity later than females (section 4.4). Females may be inseminated between 9 and 24 hours after eclosion; but males younger than about 50 hours do not attempt courtship. Most (c. 95%) males reach sexual maturity 90 hours after eclosion. Thus experimental analyses of the sexual behaviour of these flies should involve females older than two days and males older than four days to be sure that they have reached sexual maturity.

Females may remate; this was shown in section 4.5 using eye-colour mutants to differentially mark progeny of first and second matings in sulfurigaster. Drosophila sulfurigaster is a closely related species and has been used in this experiment because no genetic markers are available in nasuta or kepulauana. No evidence was found that females remate while their supply of sperm is replete. The results may be interpreted as suggesting that females remate when their sperm supply is low or empty. Remated females did not produce mixed progeny sired by the first and second males, rather, there was always a sharp change from one genotype to the other. Further experiments are required before sperm displacement can be offered as an explanation for abrupt and complete changes in

the phenotype of progeny from single females.

In section 4.6 the duration of copulation was found to be equivalent in nasuta and kepulauana; both mate for about 22.5 min. Hybrid females copulate for longer (c. 28.8 min) regardless of the type of male involved. Sperm is transferred during the first 10 min in nasuta but sometimes before 5 min. If copulation is 'forced' in either species the union lasts for a much shorter and more variable period, usually about 2 or 3 min. No sperm is transferred during forced copulations.

Only three previous publications have described the sexual behaviour of flies of the nasuta subgroup (Spieth 1969, Leroy and Eucher 1981, Lambert 1982). Of these, only Leroy and Eucher (1981) deal with acoustic signalling during courtship. They study nasuta and find two types of male sound and one type of female sound which they link to wing-vibration and body-movements. One type of sound is always made during circling when the male extends a wing and vibrates it.

In the studies presented in section 4.7 audio-visual recordings were made of nasuta, kepulauana and their hybrids, and sulfurigaster, pulaua, pallidifrons and kohkoa. Only pallidifrons among the latter species was found to produce an audible signal. The kepulauana males although vigorous with wing and body movements produce a sound so faint relative to nasuta that it could not be distinguished above background noise in sonograms.

Rather than measuring the sound made by wing-extension and vibration the wing movement itself can be quantified. This yields ample and interesting data not just for nasuta but also for kepulauana and hybrids. Furthermore, large numbers of flies may be scored for this parameter simultaneously using TRACE. The wing movement variable is called the interburst-interval (ibi). An ibi is the time interval between bursts of wing movement that impinge on the female's anterior sensors.

There is no direct evidence that the interburst-interval per se is an important signal. No electrophysiological studies of anterior female receptors have been made (cf. Ewing 1978). However, there is a significant correlation between the distribution of the male ibi variable and the distribution of

the variable to which conspecific females respond.

A male that courts a female by circling from one side to the other (Fig. 18) necessarily takes a longer time (i.e. has a longer ibi) than a male which stands directly in front and simply twists and lunges - left, right, left etc. The ibi of a male is therefore indicative of an overall courtship pattern. It may be argued that the courtship of nasuta and kepulauana differ as the result of a shift in the interburst-interval signal and response. To achieve copulation with a female that responds only to rapid interburst-intervals a hypothetical and behaviourally flexible nasuta-type male must deliver wing stimuli more frequently and the only way this is possible is if he spends all his time standing in front of the female and moving to her rear only occasionally. In this way one can imagine that the kepulauana courtship form has evolved from a nasuta form by the shift in females' response to the ibi. This interpretation involves a single elemental change in the behavioural repertoire of a putative ancestral species and in this sense it might be used to predict the direction of speciation (Kaneshiro 1980). Lambert (1984a) has speculated that divergence in SMRS may occur when a signal/receiver characteristic, like the above frequency change, evolves.

The courtship of both species have a number of features in common. Firstly, males regularly alternate between left and right wings. Secondly, when close to the female's head they are approximately at right-angles to her body (Figs 16.3 and 18.3, p. 104) and thirdly, about every 10 s they move to a position behind females and tap her abdomen. However the frequency of frontal stimulation (ibi) differs. The ibi of first generation hybrid males is an exact intermediate; in F_2 males it varies greatly, some males have the nasuta ibi, others match the kepulauana ibi, while the majority are like F_1.

The mating asymmetry is marked in this species pair (section 4.9). Female kepulauana mate almost exclusively with their own males under choice or no-choice conditions. Whereas nasuta females often mate with kepulauana males under no-choice conditions but less so under choice conditions. That

female nasuta respond to different types of male signal is well corroborated by the data presented in the second and third last columns of Table 13 (p. 90) in the section on interburst-intervals. In that table it can be seen that female nasuta mate after they have been courted by males with a variety of ibi frequency distributions whereas the reverse is true of kepulauana females.

5. SEXUAL EFFICIENCY OF F_1 HYBRIDS

'The offspring from the first cross between two pure breeds is tolerably and sometimes (as I have found with pigeons) extremely uniform, and everything seems simple enough; but when these mongrels are crossed one with another for several generations, hardly two of them will be alike... '
(Darwin 1859:81)

5.1 Introduction

Generally hybrids have an intermediate appearance. Uniformity among first generation hybrids is also characteristic (Anderson 1949). This regularity breaks down in the second generation as Darwin (in the above quote) observed: an increase in diversity in the progeny of intercrossed first generation hybrids. We now understand this to be the result of recombination of heterozygous genotypes. Similarly, hybrids of the two species of Drosophila here under study were expected to be uniform and intermediate in the F_1 and behaviourally diverse in the F_2.

In the previous chapter it was shown that nasuta and kepulauana differ in sexual behaviour. No significant differences have been reported in their anatomy (Wilson et al. 1969, Kitagawa et al. 1982). Therefore, it is the behaviour rather than the morphology which is expected to be uniform and intermediate in first generation hybrids and multiform and diverse in the second generation.

Two aspects of first and second generation hybrid courtship were studied. In section 5.2 the physical variation in the patterns of F_1 and F_2 hybrid courtship are described. In section 5.3 the efficiency (or inefficiency) with which F_1 hybrids mate with non-hybrids and siblings was investigated. Knowing how early generation hybrids behave is an important basis for the evaluation of the sexual efficiency of advanced generation hybrids.

The sexual efficiency of F_1 hybrids was measured in terms of the amount of sexual activity which precedes a mating. Only with TRACE can such data be obtained. TRACE identifies males which, for example, court often and have limited success and those which court seldom and have more success; the latter are treated as being more efficient. Mating efficiency is a measurable trait and can also be tested for uniformity and intermediacy or irregularity in hybrids.

In addition the TRACE technique also generated quantitative data about behavioural transitions. With such data it is possible to identify stages in hybrid courtship which fail to elicit positive responses. In this way it was possible to account for negative heterosis due to diminished sexual efficiency.

5.2 Description of the Courtship of F_1 hybrids

Male hybrids were aged 90+ h then placed with virgin nasuta or kepulauana females in an observation chamber (Fig. 7, p. 44) or in smaller cells on the head of a microphone (Fig. 13, p. 83) and filmed. Films were later analysed in slow motion and various parameters measured. Some F_2 individuals were also examined in this way.

First filial hybrid males have a starting-position (p. 103) which, on the face of a clock, is equivalent to 10:30 (Figs 17.1, 17.5; p. 104) or 1:30 (Fig. 17.3) while a female in the middle faces 12 o'clock. This appears not to be in agreement with Spieth's (1969:267) observation that hybrid males start at a 'diagonal position at the rear', but Spieth's terminology may differ. The starting-position is understood by me to be the position in which the male stands immediately before he starts to circle or lunge or arch. Males of nasuta and kepulauana have characteristic positions from which they commence and after which they pause between bouts. Of course, a male may arrive at a female from any direction but this is not treated by me as the starting-position. Males may also pause, sometimes for long periods (c. 30 s), at the starting-

position. Thus when Spieth writes that circling of hybrids occurs only 'for a short distance' and that 'the circling movements of the hybrids are not as extensive as those engaged in by (D. nasuta) males but more extensive than those by D. kepulauana males' we find agreement between his and the present observations. Given that nasuta starts diagonally at the rear then a hybrid whose circling path is not as extensive must start further forward as shown, for example, in Figure 17.1. Rather than being diagonally at the rear these positions (Figs 17.1 and 17.3, p. 104) are diagonal to the front.

With respect to the visible aspects of courtship the F_1 hybrids appeared to be intermediate between nasuta and kepulauana. The starting-positions were intermediate (Fig. 17.1 vs Figs 16.1 and 18.1, p. 104), and the interburst-intervals were, on average, exactly intermediate. There was an average interval of 1.3 s between bursts of frontal stimulation in kepulauana, an interval of 2.8 s in nasuta and an interval of 2.05 s in F_1 hybrids (p. 88). When Kawanishi and Watanabe (1981) investigated the interpulse-interval of melanogaster/simulans hybrids, they also obtained intermediate and uniform measures.

The acoustic signal produced by F_1 hybrids was audible and the variation in its duration, although large, was not greater than was observed among nasuta signals. To give a statistical treatment of the differences between hybrid and nasuta signals has limited value since the kepulauana signal, which represents one of the parental extremes, is inaudible. Furthermore, the signal duration was variable and depended on extrinsic factors such as the condition and position of the female, interference by other males, the available space for arching and the ambient temperature. Some of these factors have been observed to reduce a nasuta signal to half, or less, of its 'normal' period. Furthermore, copulation has been observed after signals of either short or long duration. The F_1 hybrid signal resembled a burst of the nasuta émission longue but it had a shorter duration, c. 0.75 that of a nasuta signal. An important point is that the frequency of pulses within the signal is very similar to that in a nasuta signal

(104+/-3 Hz). Comparison of the émission longue duration and the pulse frequency in the hybrid and nasuta signals indicate two trends: the F_1 hybrid signal has a shorter duration but the pulse-frequency remains similar to nasuta.

A variety of behavioural types have been observed in different F_2 males. It was possible to detect the following types: (1) the F_1 hybrid courtship-type, as described above, (2) full circling from the nasuta starting-position, with wing-extension like nasuta but no sound was produced, (3) circling like nasuta (from the nasuta starting-position) but moving faster and achieving an average interburst-interval of 1.5 - 2.0 s and producing an F_1 hybrid-like acoustic signal, (4) lunging like kepulauana but with a slower interburst-interval (2.0 - 2.5 s) and producing a pulse of sound quite unlike nasuta, (5) kepulauana-like in terms of interburst-interval but nevertheless still producing a brief pulse of sound (with a curious structure, see below), (6) nasuta-like in every respect. Individual males consistently courted in the same way. Of the above, the first was the predominant form in the F_2. Of interest is the fact that individual F_2 hybrids displaying a courtship indistinguishable from that of kepulauana were not detected in a sample of 58 F_2 hybrids from nine different populations. Whereas five F_2 hybrids were classified nasuta-like.

Figures 14.d to 14.f (p. 85) are sonograms of three different F_2 males classified in category 5 above: they lunge like kepulauana with brief intervals between lunges but they nevertheless produce a faint sound. Interestingly the sound consists of two sets of pulses produced firstly by one wing (e.g., the right wing R, Fig. 14.d) and then the other (left wing L, Fig. 14.d). Close examination of the video recording of these males reveals that the first pulse corresponds to the moment when the wing is being thrust forward and the second when it is withdrawn. It is possible that sound production occurs only when the wing is in a certain position with respect to the body (e.g., Figs 16.3 or 18.2-3, p. 104) and that when a male thrusts his wing as far forward as kepulauana (Fig. 16.2) his wing passes through that critical position on

its way forward and then again on its way back producing two brief sounds.

Lambert (1982:440) described two forms of courtship which he states are variations of nasuta courtship. But one of the forms matches the F_1 hybrid courtship and it is possible, since he was working with D. kepulauana as well, that accidental contamination of his cultures occurred. (Contamination may have occurred before this in the NDSRC labs, the source of Lambert's nasuta strains. I have found such contamination among their strains of D. sulfuriqaster). No such variation in courtship was observed in this study among any of the 19 nasuta cultures from various parts of the world (p. 49). The most telling feature in Lambert's data is the starting-position of one of the forms which he has given as the equivalent of 10:30 or 1:30. Compare Figures 17.1 and 17.3 (p. 104) with Lambert's (1982:441) Figures 3.D and 3.E. His diagram neatly sums up the courtship of kepulauana (his C), nasuta (his A and B) and F_1 hybrid (his D and E) as I have observed them, although he states that it is a summary of kepulauana and two types of nasuta courtship.

5.3 The Sexual Behaviour of F_1 Hybrids

5.3.1 Introduction

Spieth (1969) has examined hybrids between kepulauana and nasuta. He writes (1969:265) 'in all cases the (F_1) hybrid offspring were large, physically vigorous flies and the hybrid males courted vigorously.' Such positive heterosis was also observed in the present work. Do hybrid males pursue females with vigour and court them only 'feebly', do they move about more and by chance encounter more females or does their increased vigour actually result in increased mating? The studies presented below focus on measures of vigour and of general sexual activity on the one hand and mating success on the other. Of great importance in these studies are the relationships between the number of confrontations between

males and females, the actual amount of time spent signalling (with respect to the total time available), and the number of successful copulations.

5.3.2 Method

Equal numbers of hybrid and non-hybrid males were placed among hybrid and non-hybrid females in an observation chamber (Fig. 7). In one trial, called the **kep-milieu** (p. 41) trial, most females were kepulauana, in the other trial, the **nas-milieu** trial most females were nasuta (Table 17). Each trial was filmed for 30 min. The sexual interactions which ensued were scored and analysed using TRACE.

Table 17. The numerical ratio of non-hybrid to hybrid females and males in trials; kep, D. kepulauana; nas, D. nasuta and hyb, F_1 hybrid.

	males present			**females** present		
	kep	hyb	nas	kep	hyb	nas
kep-milieu	5	5	5	13	2	0
nas-milieu	5	5	5	0	2	13

Discrete stages of courtship may be identified: when males orient to the starting-position, when they move away or begin to court and when females remain still, decamp, or display 'acceptance' and copulate. There is a hierarchy of possible behavioural transitions after males orient (OB) or court (C). Important one-step transitions are given in Table 18 (p. 120). When a male courts, a female may walk away (WA), jump or attempt to fly away (FT) or she may spin around with open wings and display her readiness to mate (AX). A male was assumed to be responding positively if he proceeded from orientation (OB) to wing-extension (C) or from C to C or from C to copulation (X). Conversely if a male oriented to a female then moved away from her, it was assumed that he did not receive a positive signal.

Table 18. Types of behavioural transitions and interpretations. Abbreviations given on p. v, explanation in text.

TYPE	ACTION -- REACTION			RESPONSES	
	male	**male**	**female**	**male**	**female**
1	OB -----	OE		negative	?
2		OB ------	WA	positive	negative
3		OB ------	FT	positive	negative
4	OB -----	C		positive	?
5		C ------	WA	positive	negative
6		C ------	FT	positive	negative
7		C ------	AX	positive	positive
8		C ------	FS	positive	not negative

In Table 18 the type-1 transition is interpreted as: 'male responds negatively after Orientation Begins', types-2 and -3: 'female responds negatively after Orientation Begins', 4: 'male responds positively (i.e. he starts courting) after Orientation Begins'; 5 and 6: 'female responds negatively after Courtship' and 7 as 'female responds positively after Courtship'. The relative frequencies of these transitions experienced by hybrid, nasuta and kepulauana males in nas- and kep-female milieus form the basis of the present investigation of F_1 hybrid mating efficiency.

Results are presented in two forms: numerically in tables (Tables 19 and 20) and summarized in histograms (Figs 19 to 34). Histograms are in pairs to facilitate comparison between nas- and kep-milieu trials. The six bars on the left in each histogram (e.g., Figs 19 and 20, p. 122) may be compared between the two figures. The ratio of available hybrid to non-hybrid females is shown on the right; it was not exactly 2:13 (the ratio of females present) because while mating, females were effectively 'unavailable'. The ratio of available females is not a function of the ordinate variable in the histograms.

Histograms illustrate trends and highlight differences and similarities in behavioural-transitions. It should be noted that the number of flies involved was small, the ratio

of hybrid to non-hybrid females was biased and there were no replicates*. However, TRACE is precise and it yields abundant information about individuals and their sequence of sexual behaviour. The actions of each of the three types of male were examined in detail for 300 min (two trials, five males per type per trial, each trial for 30 min). On the one hand, then, there is an abundance of data, but on the other, it is necessary to bear in mind that the trends presented in the following pages are based on a limited number of flies.

For the purpose of presenting data graphically, they were standardized; therefore in the histograms males have the same available time (**Ta** = 70 min). The standardization of data was possible because the amount of time that each male type was able to court could be determined with precision. Of course, males were unable to court while they copulated. The time that males were available can be calculated with the equation:

$$\mathbf{Ta} = (\mathbf{Te} - \mathbf{Ti}) - (\mathbf{Tec} - \mathbf{Tsc})$$

Ta is the time available, the time during which a male was unencumbered and free to court. In the same way the time a female is unencumbered can be calculated. Since post-copular females are unattractive (Chapter 4), they were effectively unavailable between the end of copulation (**Tec**) and the end of the trial (**Te**); by making **Tec** = **Te**, for such females, a more appropriate **Ta** was calculated. Females were only available once males were present so the introduction time (**Ti**) for females is given as the **Ti** of the first male to be inserted. Males were always inserted into the arena after females and in relatively quick succession (e.g., Table 2, p. 47).

Before standardization, the **Ta** values for males of the same type (nas, kep or hybrid) were pooled (Table 19). Thus in the kep-milieu trial the kepulauana, hybrid and nasuta males

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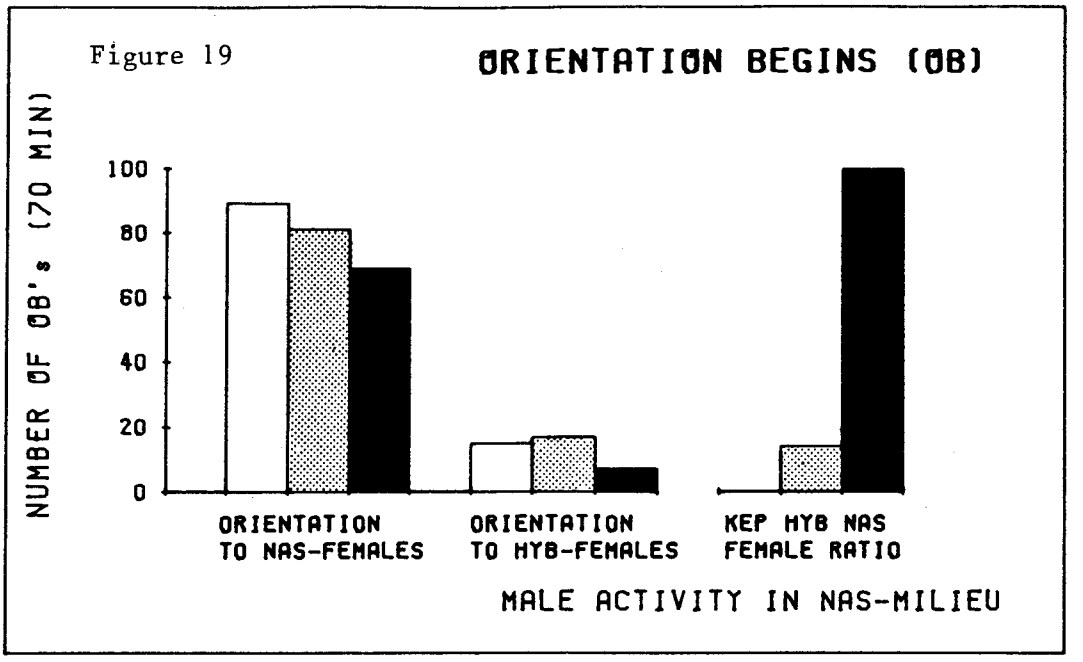
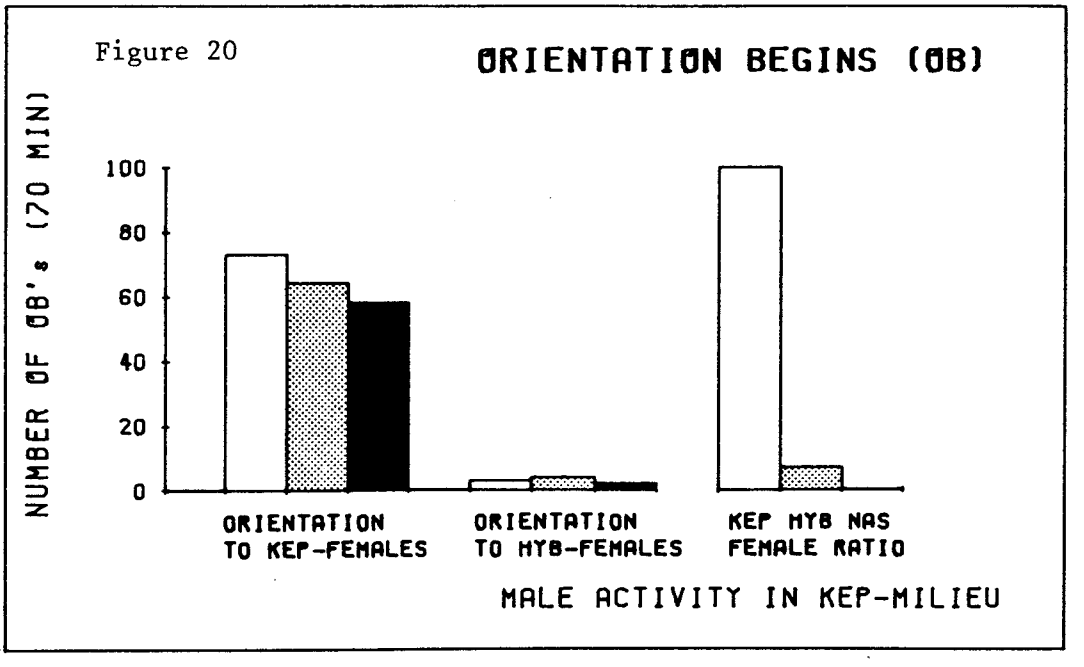
* Replicate trials were carried out and filmed but since no software was available for their analysis it was possible to manually analyse only two trials.





Figures 19 and 20. Histograms depicting the number of times males move to the starting-position (**O**rientation Begins), in nas- (Fig. 19) and kep-milieu (Fig. 20) trials; kepulauana white, hybrid stippled and nasuta black.

Before standardization the **Ta** values for males of the same type (nas, kep or hybrid) were pooled. In the kep-milieu trial the kepulauana, hybrid and nasuta males had 99, 77 and 134 minutes available to court, respectively. While in the nas-milieu trial these males, in the same respective order, had pooled **Ta**'s of 117, 68 and 93 minutes (see Table 19, below). This means, for example, that kepulauana males in the nas-milieu had almost twice as much available time (**Ta** of 117 min) as did hybrid males (**Ta** 68 min) in the nas-milieu trial. Standardization to an arbitrary 70 min allows comparisons to be made within a trial and to generate expected values in chi-square analyses.

Females are encumbered for about 22 minutes (p. 80) while mating. However, by calculating their times of availability they may be compared to other females which were unencumbered and received more courtship. The pooled **Ta**'s of like-females, in both trials, indicate that in the kep-milieu trial, kepulauana females were available for a total of 249.6 min while F_1 hybrid females for only 17.6 min. In the other milieu, nasuta females were available for 235.5 min and F_1 hybrid females for 32.5 min (see 'female ratio' in each histogram, Figures 19 to 34).

The number of wing-extensions (C's) was used to calculate the amount of time spent courting (i.e. **Tsex**, see below). In turn the amount of time spent courting was correlated to the number of female acceptance postures (AX) to give a measure of courtship efficiency. Advanced hybrid courtship efficiency has been determined on a much larger scale and in a different manner in the next chapter.

When males court they extend their wings. The number of wing-extensions is a measure of the amount of courtship but it should be noted that kepulauana males deliver many more wing-extensions per unit time than do nasuta males; F_1 hybrids deliver an intermediate number. Using the interval of time between consecutive wing-extensions (the interburst-interval, section 4.8), it was possible to calculate **Tsex:**

Tsex = (ibi) x (number of wing-extensions)

The **Tsex** values in the kep-milieu trial are more than double those in the nas-milieu trial (i.e. males spent twice as much time courting in the kep-milieu than in the nas-milieu). What, then, is the relationship between the time spent courting (**Tsex**) and the total time available (**Ta**)? A quotient (**Tq**) of these two variables was calculated:

$$Tq = Tsex / Ta$$

Thus, if the sex quotient (**Tq**) equates to unity, males spend all their time courting. High values indicate high sexual activity and low values low sexual activity. All values lie between 0 and 1. Vigorous and persistent courtship consists of a series of wing-extensions (a bout) punctuated by inter-bout pauses of variable lengths of time. The calculated value, **Tq**, will, therefore, be slightly smaller than the actual fraction of sexually-active time to total time. (N.B. if an inter-bout pause exceeds 6 s it is part of **Ta**, if less than 6 s it is an ibi variable, p. 89). Chi-square tests have been used to test for significance in observed trends.

5.3.3 Results

Bastock and Manning (1955) used the term 'orientation' to describe when a male 'takes up, and maintains, a position near the female, facing her if she is still, or following her if she is moving'. Other workers (e.g., Cobb et al. 1985) follow this usage. Orientation Begins (OB) is when a male assumes the 'starting-position'. It is always associated with legging and through legging it is possible that chemical signals are transmitted and received (p. 103).

If orientation is at random then it is expected that the frequency of orientation to the two types of female in each trial will match their relative availability. If found to be random then any deviation from randomness occurring thereafter would be due to interaction of a sexual rather than a stochastic nature.

Table 19. Raw data from the two TRACE trials: kepulauana-milieu trial, first three columns; nasuta-milieu trial, last three columns; see p. v for abbreviations and text for explanation; mal: males, fem: females.

		fem	mal kep	mal hyb	mal nas	mal kep	mal hyb	mal nas	fem
number:		15	5	5	5	5	5	5	15
Ta (min):			98.8	77.1	134.4	116.9	68.2	92.8	
Ta (% of max.):			73.5	57.4	100.0	87.0	50.8	69.1	
			kep-milieu			**nas-milieu**			
	OB	kep	106	72	115	152	81	94	nas
OB ---	OE	kep	44	13	18	62	28	34	nas
OB ---	OEX	kep	10	6	7	4	2	4	nas
OB ---	WA	kep	9	17	30	55	37	24	nas
OB ---	FT	kep	0	5	7	0	2	0	nas
OB ---	C	kep	41	27	51	23	10	22	nas
	C	kep	376	296	327	214	101	130	nas
C ---	WA	kep	7	34	42	33	33	28	nas
C ---	FT	kep	3	4	10	0	1	0	nas
	AX	kep	13	4	0	0	0	9	nas
	OB	hyb	4	4	3	26	17	10	hyb
OB ---	OE	hyb	1	0	0	6	0	0	hyb
OB ---	OEX	hyb	0	0	0	1	0	1	hyb
OB ---	WA	hyb	0	0	0	12	11	6	hyb
OB ---	FT	hyb	0	0	0	0	0	0	hyb
OB ---	C	hyb	3	3	3	5	4	3	hyb
	C	hyb	27	29	11	48	81	18	hyb
C ---	WA	hyb	0	0	1	12	11	5	hyb
C ---	FT	hyb	0	0	0	0	0	0	hyb
	AX	hyb	0	2	4	0	0	3	hyb

The number of times males orient (OB) can be partitioned into the number of times this involves hybrid (OB.hyb), kepulauana (OB.kep) or nasuta (OB.nas) females. In the kep-milieu trial **Ta**.kep:**Ta**.hyb (females) = 93.4:6.6, in the nas-

milieu trial **Ta**.nas:**Ta**.hyb (females) = 87.9:12.1. The hypothesis that male orientation is proportional to female availability can be tested using the ratios OB.hyb:OB.kep for males and the ratio **Ta**.hyb:**Ta**.kep for females and chi-square. Similarly, in the other trial, OB.hyb:OB.nas has been tested for significant deviation from the expected ratio: **Ta**.hyb: **Ta**.nas. These are 'between-sexes, within-trial' comparisons.

It was found that the orientation (OB) scores do not deviate significantly ($P<0.05$) from the null hypothesis expectation. Orientation (OB) to females was at random in both trials. Males did not orient selectively to hybrid females in preference to non-hybrids, nor *vice versa*. The number of times males orient (OB) was proportional to the availability of hybrid and non-hybrid females. This was true in both trials.

It is also possible to test the hypothesis that certain males orient more than others within a trial: a 'between-males, within-trial' comparison. The null hypothesis that male orientation (OB) is proportional to the times males have available (**Ta**) was tested using chi-square. Even though males begin in an equal numerical ratio 5:5:5 (Table 17) the effective ratio **Ta**.kep:**Ta**.hyb:**Ta**.nas (males) was 98.8:77.1: 134.4 (min) in the kep-milieu trial and 116.9:68.2:92.8 (min) in the nas-milieu trial. The 'Orientation Begins' scores for males in the kep-milieu trial was 110:76:118, and in the nas-milieu trial, 178:98:104, (kep:hyb:nas) (Table 19, p. 125).

Different types of males were found not to deviate significantly ($X^2 = 3.22$, $P<0.05$, df = 1) in the kep-trial but significantly ($X^2 = 6.41$, or 6.07 with Yates correction, $P>0.05$, df = 1) in the nas-trial. The margin of significance in the latter is not great. *Drosophila nasuta* males were expected to have an OB score of 126.8 among the *nasuta* and hybrid females (nas-milieu), but their score was only 104 (they oriented 94 times to nas females and 10 times to hybrid females, Table 19); *kepulauana* males, which had more time available (117 vs 93 min) were expected to have a score of 159.9 OB's, but the five *kepulauana* males oriented 178 times (Table 19). In both milieus *nasuta* males oriented fewer times than expected, while *kepulauana* and, to a lesser extent,

hybrid males oriented more than expected (as shown above in the kep-trial, this trend was not significant at P = 0.05). In both the kep- and nas-milieus the expected and observed orientation scores for hybrid males, were close (76 vs 75.5 and 98 vs 93.2, respectively).

A final analysis of the initial orientation scores involves an examination of the same type of male in the different milieus i.e. a 'within-male, between-female' comparison. This is, therefore, a test of the effect of the variable female type. Three chi-square analyses were made for the three types of males. The total number of OB's scored in the kep-milieu and in the nas-milieu were compared to values which would be expected if the scores were dependent on the time males had available (**Ta** males). For example, the OB scores for kepulauana males in the two trials were 110 and 178, the **Ta** scores were 117 and 99 min, therefore the expected scores were 131.9 and 156.1.

Chi-square results were significant ($P<0.05$) for kepulauana and hybrid males but not for nasuta males. Drosophila kepulauana males oriented less often in the kep-trial and more than expected in the nas-trial; the same was also true of hybrid males, but the trend was less pronounced. When surrounded by many nasuta females males tended to orient frequently, moreso than when the predominant female was kepulauana.

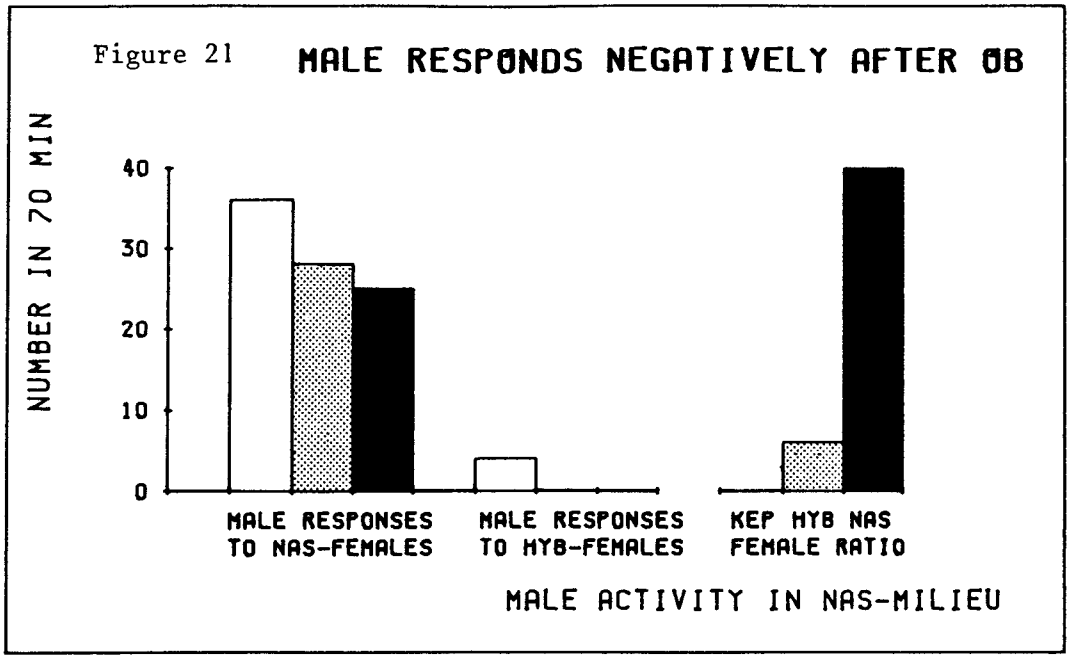
Figures 19 and 20 show that orientation was at random and proportional to the availability of females. In the kep-milieu trial the number of times orientation begins was fewer than expected but still random. The rarer hybrid females were apparently not more attractive thus they did not draw more attention from males. The hybrid males oriented an intermediate number of times in both milieus but also at random.

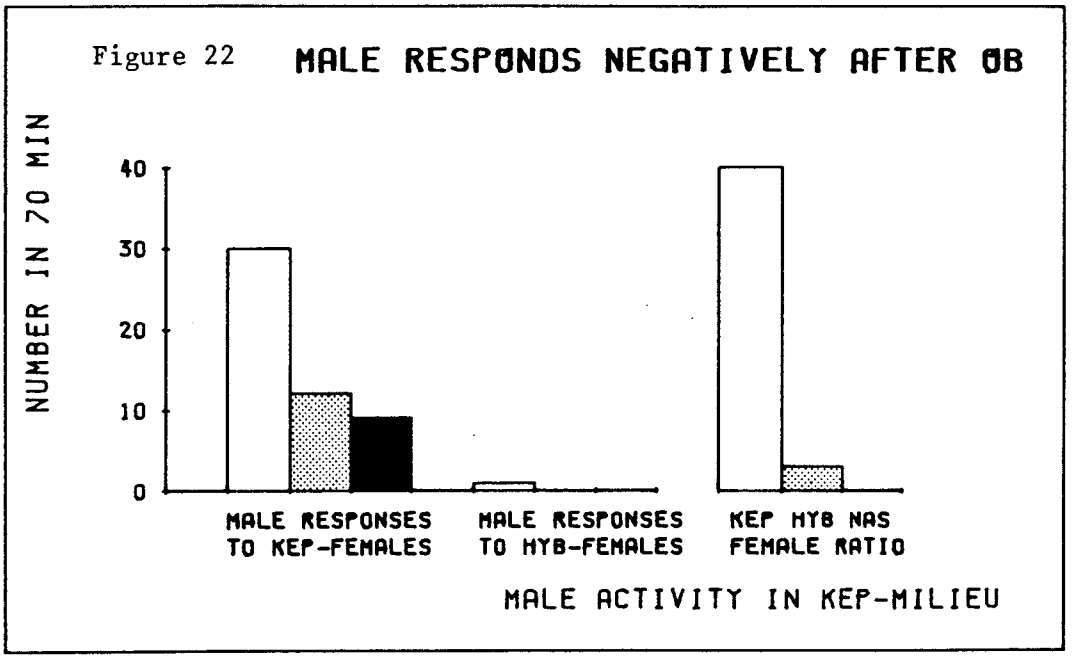
Male Responds Negatively After Orientation Begins (OB). In Swaziland, Drosophila nasuta males were observed orienting to other drosophilid species in nature. They responded negatively when the other fly was not a female nasuta i.e. they

'broke off' or terminated orientation by moving away. The behavioural element 'Orientation Ends' (OE, voluntary termination by males) was scored here. The transition OB followed by OE was interpreted as 'male responds negatively after orienting to a female' (Table 18, p. 120). It is implied here that when a male walks away from a stationary female to which he has just oriented, he is responding negatively to her signal. Likewise if she moves away from him she is responding negatively to his signal.

The results may be viewed in two ways. The number of transitions, OB - OE, may be compared either to the total OB score (less the number of terminations caused by an extraneous factor, OEX) or to the amount of time available. For example, kepulauana males oriented 106 times in the kep-trial and 152 times in the nas-trial (Table 19). The transition OB - OE (involving non-hybrid females) occurred 44 times in the first and 62 times in the second trial (upper sections Table 19). Thus kepulauana males terminated orientation with kepulauana females about 46% of the time (i.e. 44/(106-10)%) in the kep-milieu and 42% of the time in the other trial (Table 20, p. 135). However, one could also relate the number of transitions to the times available i.e. 44 times in 98.8 min in one trial and 62 times in 116.9 min in the other (Table 19). I consider it more informative to relate the transition to the quantity of the first element (OB) less the number of disturbed orientations (OB - OEX). The upper sections of Table 19 were examined for trends within males and between females and the reverse. Chi-square was used and expected values were based on the proportions of OB less OEX.

Drosophila kepulauana males end orientation c. 46% of the time after orienting to kepulauana females; hybrid males end orientation only 20% of the time and nasuta males make the transition from orientation begins (OB) to orientation ends (OE) only 17% of the time; the latter percentages are small. However, Table 20 (p. 135) shows that orientation by alien males to kepulauana females was more often terminated by the female; they moved away more often from alien males than from their conspecifics (see below). When kepulauana males oriented





Figures 21 and 22. Histograms depicting the number of times that males move away after Orientation Begins; in nas- (Fig. 21) and kep-milieu (Fig. 22) trials; kepulauana white, hybrid stippled and nasuta black.

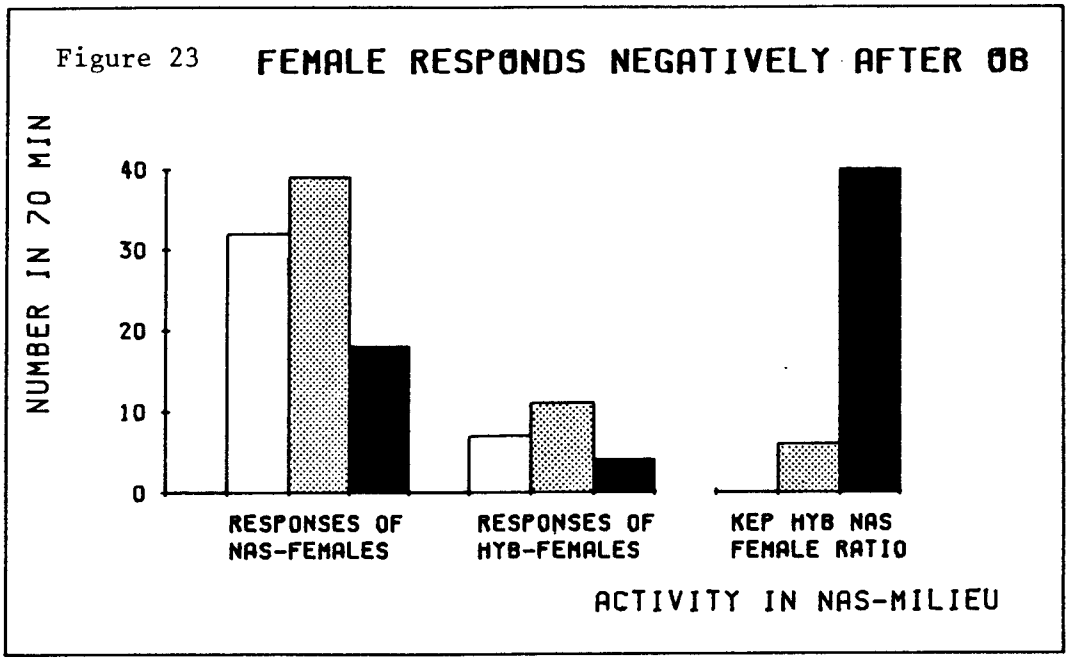
to nasuta females the OB - OE transition occurred 42% of the time (cf. 46% in the other trial), the score for hybrid males was 35% and for the nasuta males, 38%. Are these significant?

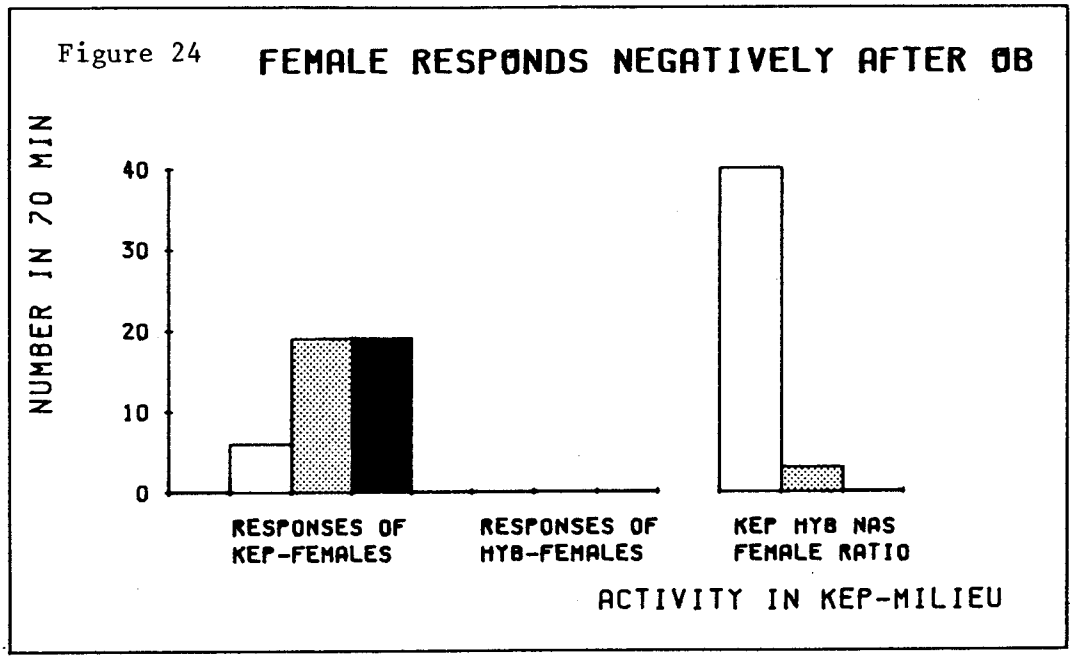
In the kep-milieu the differences in the number of times males terminated orientation (OB - OE) is significant (X^2 = 17.6, P<0.001, df = 2) when compared to their OB scores; kepulauana males terminated orientation with kepulauana females more often than other males, while nasuta males did so much less often than expected. In the nas-milieu trial there was no significant deviation from the null hypothesis expectation that the transition, OB to OE, is proportional to the OB (less OEX) scores (X^2 = 0.6, P>0.05, df = 2). All males behave in a similar way in the nas-milieu but not in the kep-milieu.

A 'between-trial within-male' analysis reveals that the behaviour of kepulauana and hybrid males to nasuta and kepulauana females was similar with respect to the number of times the transition OB - OE occurred (X^2 = 0.2 (kep), X^2 = 3.15 (hyb), P>0.05, df = 1). Not so for the nasuta males which terminated orientation significantly (X^2 = 8.33, P<0.01, df = 1) more often with their conspecific females compared to kepulauana females (Figs 21 and 22).

The results concerning hybrid females are too scant for comment. A trend which might, with further study, be found to be significant, was that hybrid females were seldom involved in OB - OE male transitions i.e. males stayed with hybrid females until the female moved away. Males did not seem to cause termination of orientation to hybrid females themselves.

Female Responds Negatively After OB. If a female moved away after Orientation Begins her response was treated as negative. The behavioural transition discussed here is OB followed by either 'Walking Away' (WA) or 'FlighT evasion' (FT) (Tables 18 and 19, Figs 23 and 24). Like the orientation ends (OE) element the number of negative responses is best viewed in context to the number of OB's (cf. **Ta**'s). For these calculations no distinction will be made between the elements WA and FT so scores are combined.



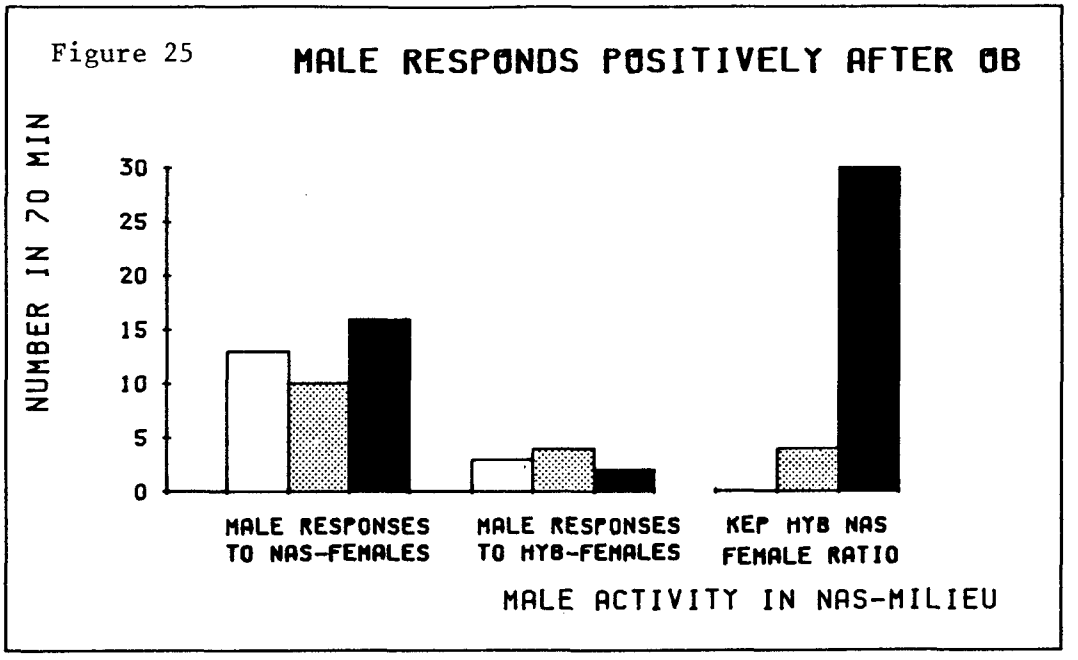


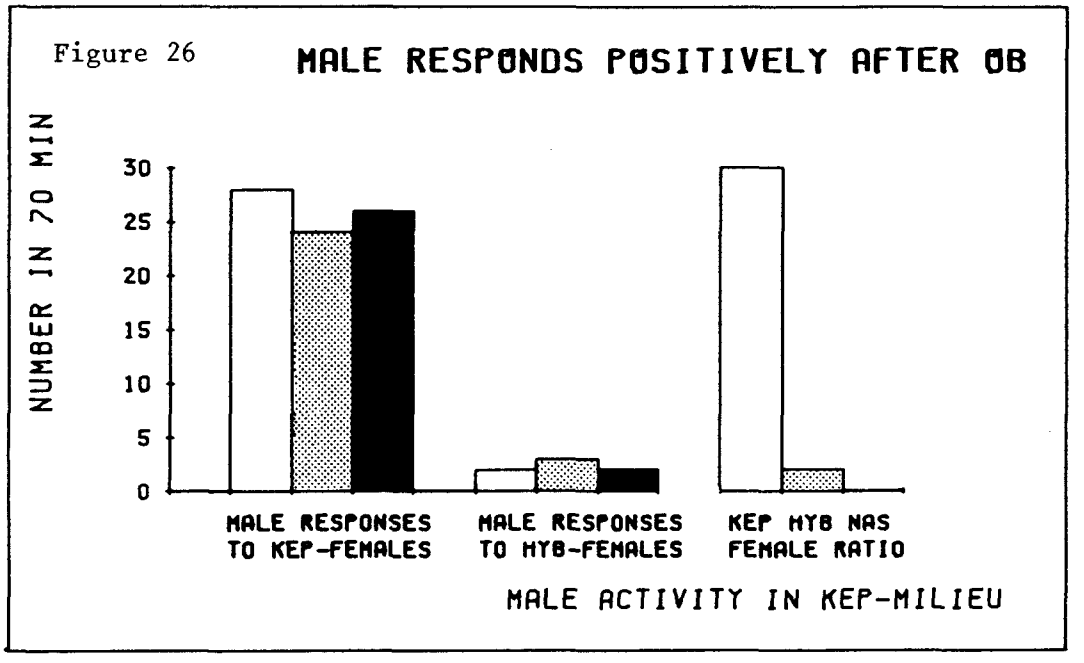
Figures 23 and 24. Histograms depicting the number of times that females move away after Orientation Begins, in nas- (Fig. 23) and kep-milieu (Fig. 24) trials; kepulauana white, hybrid stippled and nasuta black.

In the kep-milieu trial (raw data: left half Table 19, p. 125; % data: Table 20, p. 135) kepulauana females moved away after orientation began (OB) 9.4% (9+0/(106-10)) of the time from kepulauana males, 33.3% from F_1 hybrids and 34.3% from nasuta. In the other trial, with nasuta and hybrid females, kepulauana males received a negative response from nasuta females 37.2% of the time, F_1 hybrid males received a negative response 49.4% of the time and nasuta males received a negative response 26.7% of the time (Table 20). The results for hybrid females are scant and not given as percentages; their negative responses appear to be proportional among male types i.e. hybrid females gave the negative response indiscriminately. It is noteworthy, however, that they terminated courtship with walking away (WA) or flight evasion (FT) much more often than do nasuta or kepulauana females. In the nas-milieu, for example, they responded by moving away 46% (from kepulauana males), 65% (from hybrid males) and 60% (from nasuta males) (Table 20, p. 135).

Drosophila kepulauana females respond in different ways to different males after orientation begins (χ^2 = 14.8, P<0.001, df = 2). They rarely respond negatively to kepulauana males. The OB - WA/FT transitions were more frequent for hybrid and nasuta males; nasuta males received negative signals from kepulauana females more often than did kepulauana males, after OB. In the nas-milieu the same trend occurred (χ^2 = 5.8, P<0.05, df = 2), nasuta females moved away from hybrid and to a lesser extent from kepulauana males, after orientation, more often than from conspecifics (Table 20).

Hybrid males received similar responses from both types of non-hybrid females (χ^2 = 2.2, P>0.05, df = 1). Female nasuta moved away from them, after OB, slightly more often than did kepulauana females. D. kepulauana males received significantly different responses from the two types of female (χ^2 = 17.1, P<0.001, df = 1), conspecific females decamped less often. However, there was no such trend associated with nasuta males when they oriented (χ^2 = 0.9, P>0.05, df = 1); the sum of the transitions OB - WA and OB - FT is proportional to the OB scores for nasuta and kepulauana females (Table 19).





Figures 25 and 26. Histograms depicting the number of times that a wing-extension occurs after orientation begins, in nas- (Fig. 25, above) and kep-milieu (Fig. 26) trials; kepulauana white, hybrid stippled and nasuta black.

Male Responds Positively After OB. If a female does not move away when a male orients toward her, the male may then begin a wing 'display' (Courtship, C). To avoid implying that the this is a visual signal (a possibility, but one not easily distinguished from a tactile or acoustic communication) I simply call it wing-extension (cf. wing-display).

Wing-extension is one of the most conspicuous aspects of the sexual interaction of D. nasuta, D. kepulauana and their hybrids. The number of wing-extensions is an indication of the quantity of courtship. Unlike Drosophila species which hold wings out for longer and variable intervals of time the flies studied here make discrete wing movements - wing out, circle or lunge, wing back, other wing out, circle or lunge, and so on throughout a bout. During TRACE analyses a C (wing-extension) is scored when a male, with his wing extended, moves to - or passed - the front position (Figs 16.3, 17.2 and 18.3, p. 104). Results are presented here of a score of the transition OB - FS - C; male orients, Female remains Stationary (FS), male courts (C); it is equivalent to OB - C. This is interpreted as 'male responds positively after Orientation Begins'. The FS (female stationary) action may be a neutral signal or a positive one; here I am simply interested in the way males respond after orientation begins. Do males always proceed to wing-extension if females 'co-operate'?

In Table 19 scores of the various actions that follow OB are given. There is a baseline value (number of OB's), there is the number of times males move away (OB - OE transitions), the number of times orientation is disturbed by an extraneous factor (OB - OEX), the number of times females move away (OB - WA and OB - FT) and the number of times males begin to extend their wings (OB - C). Here the proportion:

$$(OB\text{--}C) \,/\, ((OB) - (OB\text{--}OEX)) \times 100$$

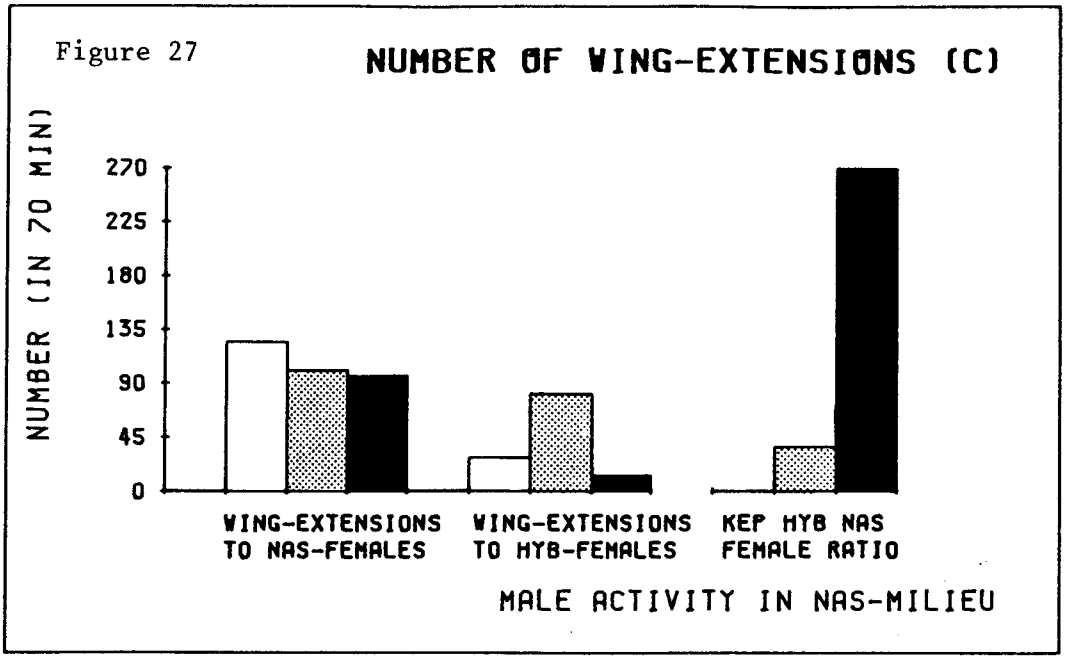
is given as a percentage (Table 20). This indicates the relative number of times that a male is positively stimulated. It is interesting to compare these percentages to the frequency of negative responses (OB - OE).

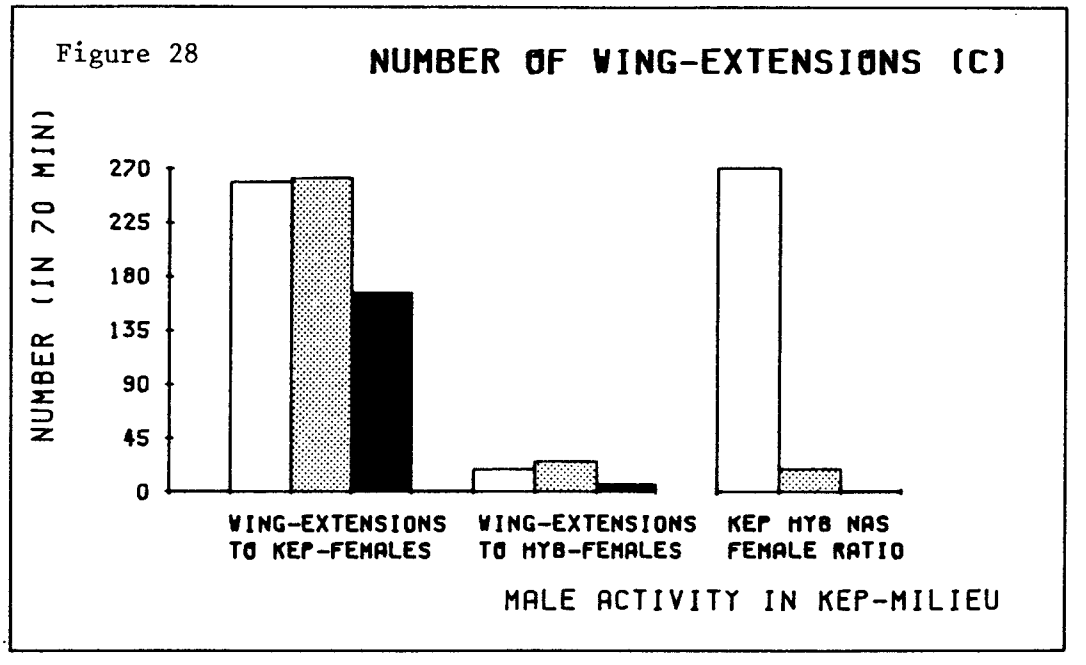
Table 20. Frequency of transitions after OB. The three transitions are equivalent to male responds negatively (a), female responds negatively (b), male responds positively (c), after OB. The remainder (d) cannot be assigned to any of the categories a - c.

FEMALES	MALES			MALES			TRANSITION
	kep	hyb	nas	kep	hyb	nas	
nas				41.9	35.4	37.8	a OB -- OE
nas				37.2	49.4	26.7	b OB -- (WA+FT)
nas				15.5	12.7	24.4	c OB -- C
nas				5.4	2.5	11.1	d
kep	45.8	19.7	16.7				a OB -- OE
kep	9.4	33.3	34.3				b OB -- (WA+FT)
kep	42.7	40.9	47.2				c OB -- C
kep	2.1	6.1	1.8				d
	100.0	100.0	100.0	100.0	100.0	100.0	OB

Drosophila kepulauana males proceed to courtship often (42.7%) with conspecific females and seldom with alien females (15.5%). However, nasuta males make the OB to C transition 24.4% of the time with nasuta females and **more** often (47.2%) with kepulauana females. Hybrid males start 'winging' after orientation often (40.9%) in the kep-female milieu but seldom (12.7%) in the nas-milieu. There is a striking similarity in the way nasuta and hybrids behave in the kep-milieu.

For the significance of these proportions the OB scores are used to generate expected values and chi-square analyses are made of OB - C transition scores. All males behave in a similar way among kepulauana females (X^2 = 0.4, P>0.05, df = 2) and among nasuta females (X^2 = 3.9, P>0.05, df = 2). D. kepulauana males proceed to wing-extension after orientation begins significantly more often when they have oriented a conspecific female (X^2 = 16.4, P<0.001, df = 1), so too do nasuta males (X^2 = 6.9, P<0.05, df = 1). Hybrid males make the OB - C transition more often than expected with kepulauana females (X^2 = 11.3, P<0.001, df = 1) than with nasuta females (Figs 25 and 26).





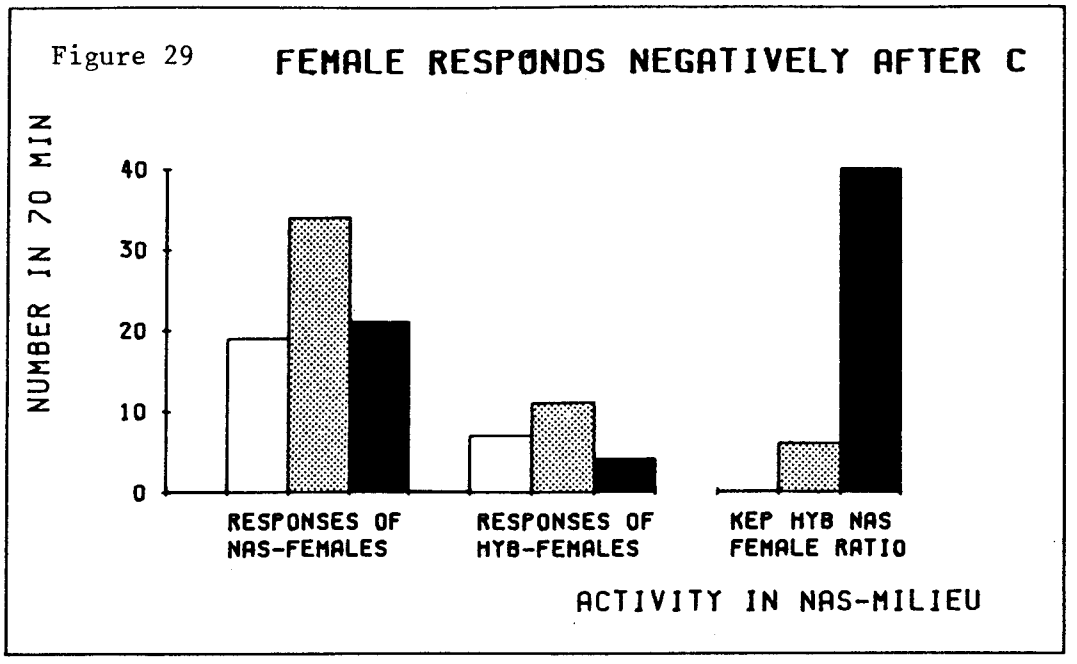
Figures 27 and 28. Histograms depicting the number of wing-extensions delivered by males, in nas- (Fig. 27, above) and kep-milieu (Fig. 28) trials; kepulauana white, hybrid stippled and nasuta black.

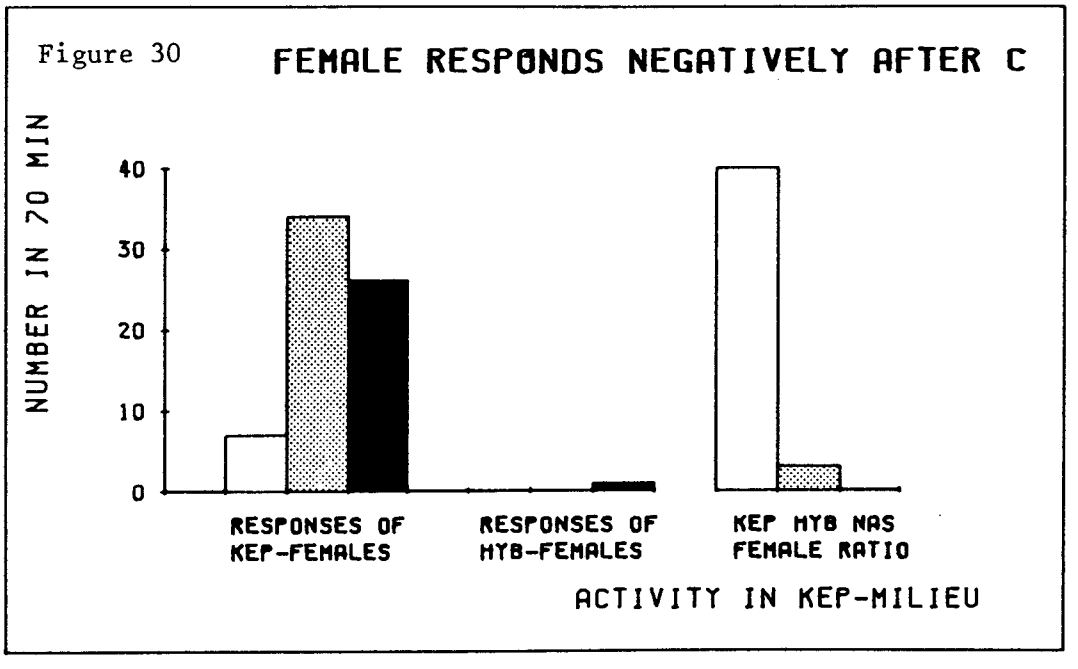
Number of Wing-Extensions (C). The number of times a male extends his wing can be used to calculate the time that the male actively engages the female during courtship. This is because in a separate study, the results of which have been presented in section 4.8, the time interval between wing-extensions was calculated for each type of male. The sexually active time for a male is the product of the interburst-interval and the number of wing-extensions (p. 123). It should be borne in mind that the number of wing-extensions must be related to the total time available (**Ta** of a male) and the interburst-interval of that male-type.

Table 19 lists the scores for this variable (C). The histograms in Figures 27 and 28 show the relative number of wing-extensions (C's) in a standard time (70 min). From the table and figures it can be seen that there is much more sexual activity when males were among many kepulauana and a few hybrid females than when they were among nasuta and hybrid females. It is also interesting to note that hybrid males deliver almost as many (C's) to hybrid females as they do to nasuta females (Fig. 27, Table 19) even though the effective ratio of females is 87.9:12.1 (i.e. **Ta**.nas:**Ta**.hyb, see right columns of Fig. 27) in the nas-milieu. This trend was completely absent in the kep-milieu trial (Fig. 28).

For statistical analyses the **Ta** values (Table 19) were used to generate theoretical values. The null hypothesis was that the number of wing-extensions for a particular type of male, within a trial, are proportional to the time available for those males. It was found that all males deliver many more wing extensions than expected to kepulauana females than to nasuta females (χ^2 = 76 (kep males), χ^2 = 73 (hyb males), χ^2 = 29.1 (nas males), $P<0.001$, df = 1).

Female Responds Negatively After C. If a female is receptive to courtship she does not move away; moving away does not result in copulation and often disorients males. But if she does not move and courtship proceeds she may display her 'readiness' to mate, by spinning around and by spreading her wings in the 'female acceptance' (AX) posture. Remaining



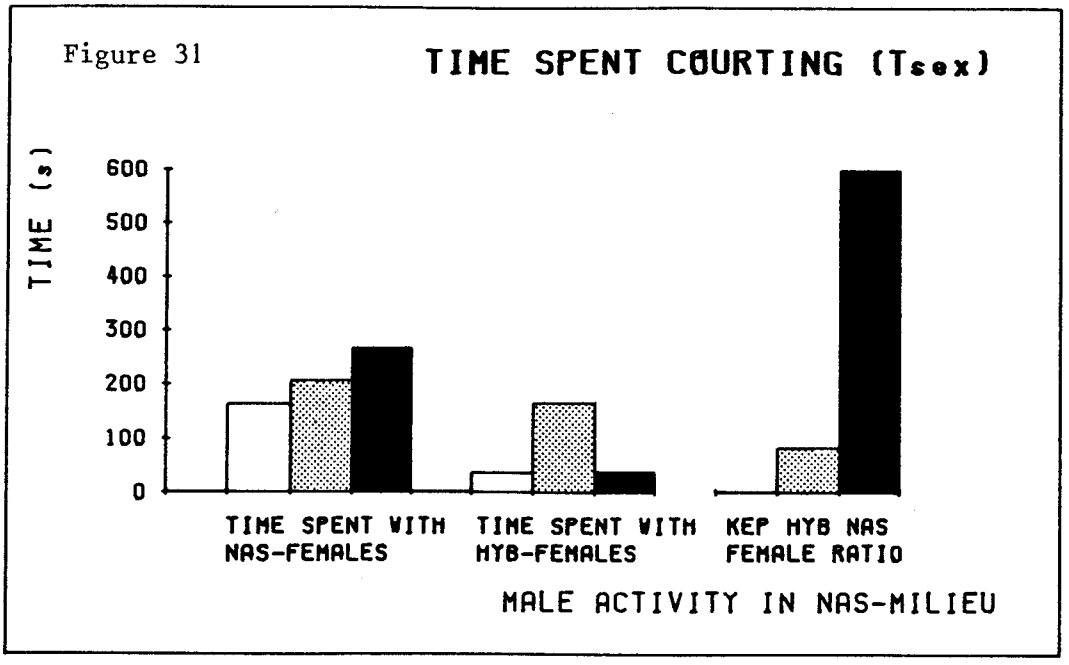


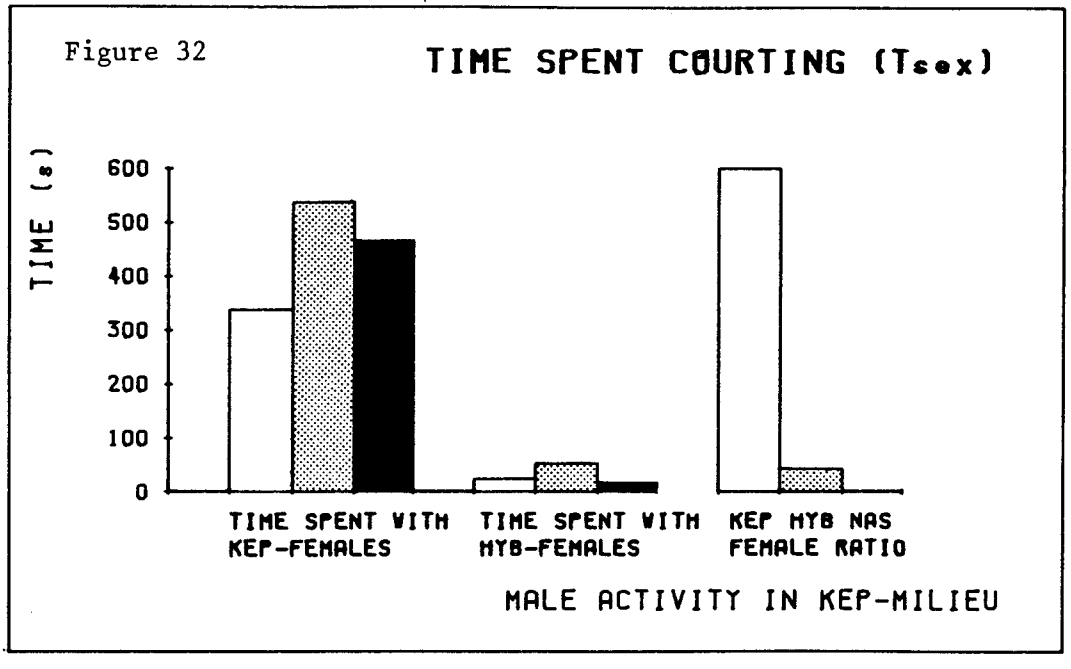
Figures 29 and 30. Histograms depicting the number of times a female moves away after a male's wing-extension; in nas- (Fig. 29, above) and kep-milieu (Fig. 30) trials; kepulauana white, hybrid stippled and nasuta black.

still (FS) and the acceptance display (AX) are both non-negative responses whereas walking away (WA) or flight evasion (FT) is a negative reaction (FS could also be regarded as neutral, this is why I do not say 'positive') . Here the behavioural transitions C - WA and C - FT are combined for statistical analysis.

The null hypothesis is that if the number of negative female responses is randomly distributed and independent of the type of male then the number of the transitions (C - WA+FT) should be proportional to the amount of time spent by males courting (**Tsex**, p. 123). By generating expected values for a chi-square test in this way it was found that there were significant trends present in both trials. Firstly, the number of times that kepulauana males were involved in the C - WA+FT transition with kepulauana females was far fewer than expected. Secondly, the observed number of times that kepulauana females moved away from hybrid and nasuta males after they courted or 'winged' them was more than expected. In simple terms kepulauana females move away from F_1 hybrid and nasuta males significantly more often than they do from their conspecific males after wing-extension (χ^2 = 11.5, P<0.01, df = 2). Exactly the same trend is observed in the other trial i.e. nasuta females move away from their conspecific males less often than they do from the hybrid and kepulauana males (χ^2 = 9.14, P<0.05, df = 2) (Figs 29 and 30, see also Table 20, p. 135).

Time Spent Courting (Tsex). As far as is known there is no other technique available that so accurately partitions the amount of time Drosophila males, of different types, spend courting different females while held together in the same chamber. The results are interesting. Figures 31 and 32 show that the amounts of time spent courting by the different males are variable. Note that hybrids spent a large part of their sexually active time courting hybrid females in the nas-trial. Also of interest were the extended periods of time spent by hybrid and nasuta males courting kepulauana females; but this was to no avail (see below). A test of the significance of





Figures 31 and 32. Histograms depicting the total time spent courting by males in nas- (Fig. 31, above) and kep-milieu (Fig. 32) trials; kepulauana white, hybrid stippled and nasuta black.

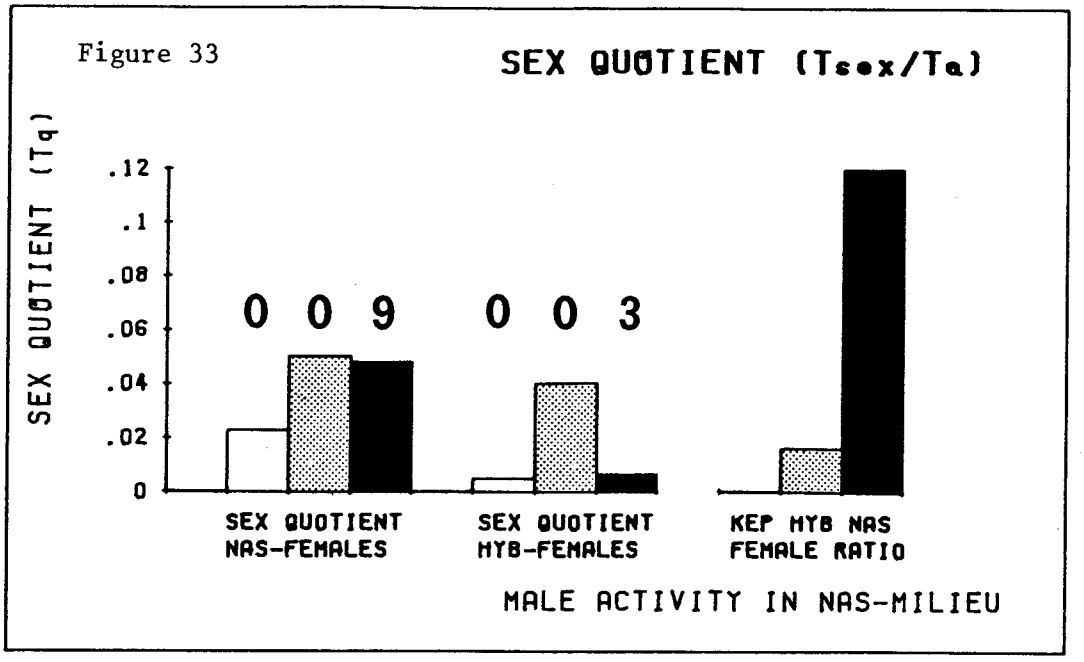
this variability rests on a determination of the proportion of the total time available and the time spent courting, this is **Tq**, the sex quotient, discussed further below.

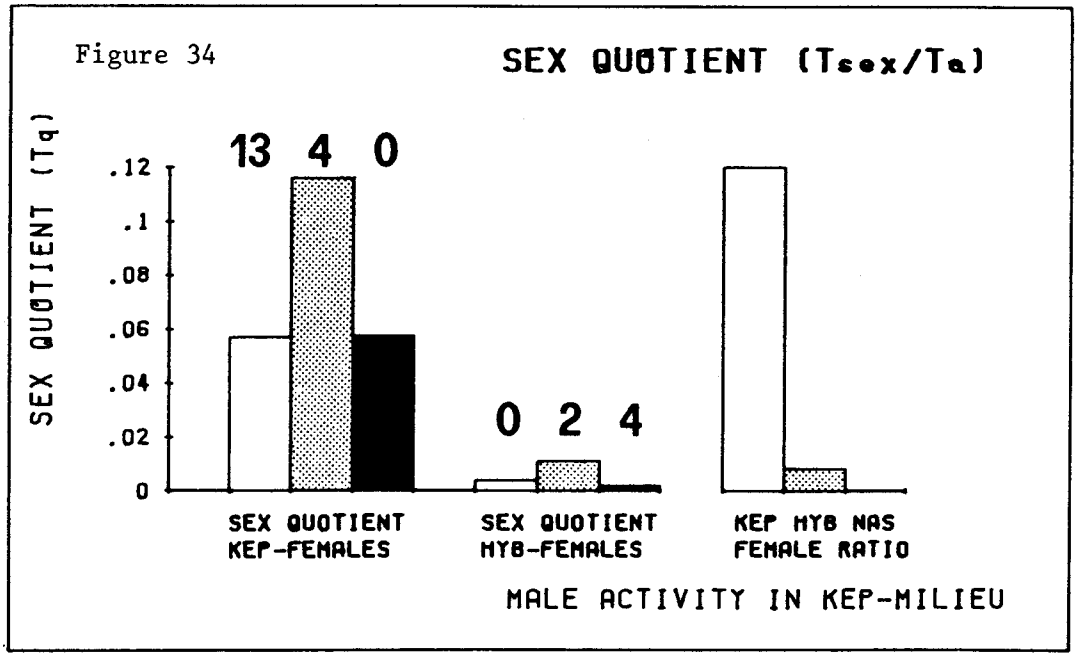
Sex Quotient (Tq). The sex quotient (**Tq**) is the proportion of total time available to a male and the time he is sexually active (p. 124). A sexually active male will have a high quotient and an inactive male will have a low quotient. All quotients measured here are in the range 0.02 to 0.12 but given that males spend much time moving around locating females before they court them, this is not surprising. A **Tq** of 0.02 indicates that in a 30 minute trial, for example, a male courts for c. 0.6 min.

A comparison between Figures 33 and 34 reveals some interesting phenomena. Hybrid males spent a lot of time courting relative to non-hybrid males. In the nas-milieu the sex quotient is 0.06 for kepulauana males, 0.13 for hybrids and 0.12 for nasuta.

In the nas-milieu, hybrid males spent half their sexually active time courting hybrid females even though the availability of these females was significantly less than the other females (**Ta**.nas females, 235.5 min; **Ta**.hyb females, 32.5 min). However, they failed to induce any females to copulate (see numbers in Figs 33 and 34) whereas the 5 nasuta males received 9 positive signals (female acceptance, AX) after courting nasuta females. The kepulauana males, by comparison, did not spend much of their time courting, they did not receive AX signals from nasuta females in the nas-milieu.

In the other trial with many kepulauana females and few hybrid females the difference in the activity of males is even more striking. Once again, hybrid males spent relatively much (0.09) of their time courting whereas the quotients for kepulauana and nasuta are 0.03 and 0.05 respectively. The hybrid males did have some success with kepulauana and hybrid females in the kep-milieu. But an important observation was made about the four copulations with the kepulauana females: although these females spread their wings (AX), copulations were of short duration (<4 min); this has previously been





Figures 33 and 34. Histograms depicting the proportion of the total time available and the time spent courting, in nas- (Fig. 33, above) and kep-milieu (Fig. 34) trials; kepulauana white, hybrid stippled and nasuta black. The number of times courtship was successful i.e. the number of acceptance postures (AX) given by females, is indicated above each bar.

found to be associated with partial or incomplete intromission and no - or very reduced - transmission of sperm (p. 78). Clearly there is a significant difference in the number of acceptance postures that kepulauana females display to kepulauana and nasuta males despite the fact that nasuta court for longer (Fig. 32).

5.3.4 Discussion

The behaviour of F_1 male hybrids of Drosophila kepulauana x D. nasuta is uniform. They invariably move to an intermediate starting-position diagonally to the front when they orient to a female. This position may be viewed as intermediate between kepulauana males which stand directly in front and nasuta males which stand diagonally at the rear (Figs 16-18, p. 104). No F_1 male was seen to occupy a different starting-position. In contrast, F_2 hybrids have been observed in all three starting-positions, most frequently they start where F_1 hybrids start and least frequently they start in front, like kepulauana.

The courtship bout of these three male-types have the following sequence in common: (1) leg extension and leg touching (between and within sexes); (2) orientation and movement to a starting-position; (3) wing-extension; (4) body moves to a right-angular position directly in front of, and close to, female head (the costa of the extended wing is very close to the female head at this stage); (6) a sound may be produced at this point (except in kepulauana); (7) males return to their starting-position (or equivalent but opposite position). After 7, the sequence starts at 3 again and the male extends the other wing. The most distinctive visible difference between kepulauana, F_1 hybrid and nasuta courtship seems to be the time interval that occurs between stages 3 and 7. Whether the starting-position difference is independent of this time is not entirely clear for it could be argued that if a male 'is genetically programmed' to signal to a female's head region with one wing and then the other, in quick

succession, he must start close to her anterior end. In this way the ibi and the starting-position, and therefore the whole structure of the courtship pattern or sequence, is interrelated. A change in timing automatically changes the whole physical structure of the behaviour pattern.

The interburst-interval was measured by scoring the time between the consecutive moments when element 4 (above) is observed. Females, when courted by kepulauana males, receive a frontal signal, on average, every 1.3 s but every 2.8 s when the male is nasuta. The bouts, for both species, last for an average time of c. 10 s; thus kepulauana males may extend their wings about 6 or 7 times during a bout whereas nasuta males do so less often. The ibi of hybrid males is exactly intermediate (2.05 s) and this is good evidence of the polygenic nature of this parameter. It is also more uniform than the F_2 ibi as indicated by the greater spread in the F_2 distribution (see Tables 12 and 13 p. 89).

The association between the timing characteristic (ibi) of a male's signal and mating success was determined by examining the timing characteristics of signals received by females just before they respond positively. In other words, during a TRACE trial, rather than tracking males and scoring the intervals between wing-extensions, females were tracked, and the interburst-intervals that they receive in the 30 s just prior to when they give the acceptance signal was scored (section 4.8). It was found that female kepulauana respond to male signals which are punctuated by an interburst-interval like that of kepulauana males. D. kepulauana and hybrid 37.F_{23} males were shown to have a similar time-class distribution of the ibi variable; other male-types differ significantly ($P<0.001$) (Table 13, p. 90). It was then shown that the time characteristics of the signals to which kepulauana females are responsive match only these two distributions and not the others. In contrast, it was found that nasuta females were responsive to various time-class distributions of male signal. D. nasuta, 26.F_1 and 26.F_2 males court with a different 'tempo', the distributions of the ibi variable are significantly different ($P<0.001$). Yet, it was found that the

type of signal to which nasuta females were responsive did not differ significantly from any one of these three different distributions (Table 13). Furthermore, it was found that the signal pattern (and by this I mean the distribution of the ibi variable) of F_1 males does not match the response pattern of their sibling hybrid females ($P<0.05$). Thus, with respect to this very important parameter of courtship, which is dependent upon the physical movement and frequency of actions, there is good evidence that first generation hybrid males are not coadapted to their sibling females. Moreover, these F_1 hybrid females are not responsive ($P<0.001$) to signals which have a time characteristic or 'tempo' like that of the average nasuta or kepulauana male.

In this chapter it has been shown that orientation occurs at random. This is independent of the type of female or male involved. No detailed results are available when males were among both nasuta and kepulauana females. In these studies males were placed only among hybrid and one parental kind of female. The number of times a male oriented to a female was directly proportional to her availability. It is as if the males moved around at random and oriented whenever they happened to 'bump into' a female.

Males oriented only to females but, of course, they often 'bumped into' other males. What sort of signal allows males to identify the sex of another fly? When flies are in close proximity with one another they extend their legs regardless of sex. Legging is always observable when flies are moving close to each other. It appears that through legging a male is able to determine the sex of another fly. Furthermore, it appears that he is able to determine whether or not a female is inseminated; males switch from relatively passive to highly excited states after they leg virgin females (the possibility that another short-range channel of communication is operating, is not denied). It is possible that through legging, information may be transmitted. If so, the most likely cause of sexual excitation in males is the reception of a chemical signal, perhaps hydrocarbons on leg cuticle. The existence of a cuticular hydrocarbon which acts like an

'aphrodisiac' (Jallon et al. 1986) is probable. Among the species under study here, and their hybrids, there is circumstantial evidence that something (most probably a chemical signal) gives information about sex and the state of fertility when males leg females. The nature of this signal is apparently the same or similar in kepulauana and nasuta females (and therefore in hybrids) because after legging orientation occurs regardless of male or female type; but it occurs only when males leg females not when they leg other males.

Orientation to females is at random but the outcome i.e. the next event, is quite different and is highly dependent on the type of male and female involved. Other types of signals are implicated at stages in courtship after legging and orientation.

First filial hybrid males are stimulated by signals from nasuta and kepulauana females because after orientation they frequently proceed to courtship. However, before this transition it is just as likely that females will move away from them because it was shown (Table 20, p. 135) that the transition: 'male orients then female moves away' is disproportionately high when hybrid males are involved. Furthermore, once hybrids have courted (extended their wing and arched across the female's anterior end) it was shown that females moved away from them significantly more often than expected. The number of times females move away from their conspecific males after courtship is significantly less.

The method used in this study is new because it allows one to measure the amount of courtship and to compare this to mating success. The amount of courtship (**Tsex**) is given as a quotient of the total time available (**Ta**) and is called the sex-quotient (**Tq**). Interpretation is difficult because on the one hand if **Tq** is low this may mean that the males and females communicate well and that males quickly respond to a negative signal from females but on the other hand it may mean that they are the opposite i.e. not well attuned. The same applies to high **Tq** scores. Information about the number of successes is important here for if **Tq** is low and a female's 'readiness

to mate' (AX) scores are high it can be concluded that the males are very well coadapted to the females. The **Tq** scores for hybrid males are the highest, they are more than double the **Tq** scores of nasuta and kepulauana males in both female milieus (Figs 33 and 34). But the number of times females are positively stimulated (by hybrids) to the point of showing their readiness to mate (AX) is very much lower.

Drosophila nasuta males achieve a remarkable ratio of **Tq** and AX with hybrid females. In both trials nasuta males spend an extremely small fraction (**Tq**<0.005) of their total time (**Ta**) courting hybrid females and yet they achieve considerably more 'female acceptances' than do the other males.

It can be concluded that despite the vigorous sexual activity of F_1 hybrid males, they have very limited success with any female. In contrast, D. kepulauana males and females seem to be the most coadapted because the lowest Tq scores are associated with the highest AX scores.

The experiments have successfully demonstrated that despite persistent courtship, hybrid males receive a disproportionate (excess) number of negative responses from females. In the end they achieve no matings with nasuta females and very few with kepulauana females. Having received the AX signal from these latter females they actually failed to copulate properly.

F_1 hybrids are uniform and intermediate, they are shown to be inefficient courters; they court for longer periods of time but achieve fewer copulations than either of the parental males. F_1 hybrid females respond more often to nasuta males than to kepulauana males (but this result is tentative, being based here only on several individuals. In the next chapter further, more substantial, evidence is found confirming this conclusion). D. nasuta females respond to a wider variety of signals than kepulauana females. Second generation hybrids are shown to have courtship signals which vary from nasuta-like to kepulauana-like with the majority remaining F_1 hybrid-like.

The populations derived from these hybrids are not artificially selected by manipulation of proportions. It is important to note that in the present study in contrast to

experiments on negative heterosis and disruptive selection (e.g., Koopman 1950, Crossley 1974, Kessler 1966), the parity of certain behavioural phenotypes is not artificially maintained by removal of heterotypes. Thus the second and subsequent filial variability exists in a state of natural flux providing the raw material upon which natural selection acts.

The complex behaviour which occurs prior to copulation may be viewed as a mechanism orienting organisms to facilitate copulation. The reduced ability to reach copulation and fertilization has, of course, the most serious consequences with respect to the fitness of motile sexual animals. Males and females which are sexually coadapted, i.e. the sensors in one sex are tuned to the signals of the other, are more likely to copulate than those between which there is little or no recognition. If sexual behaviour is indeed a kind of behavioural 'volley' involving specific signals and responses passing to and fro between potential mating partners and also if vollies leading to copulation involve many positive responses then it is most unlikely that, by chance, males and females reach copulation without having broadly complementary arrays of signals and sensors.

It was found that F_2 hybrid flies have a variety of sexual behaviours therefore it may be predicted that they will also vary in their mating success or efficiency. It may be predicted that, by chance, some male and female hybrids will have a greater portion of a whole sexual signalling system in common than others. If such flies are the ones that reproduce the most, then these individuals make the greatest genetic contribution to following generations, they will be selected. So increased coadaptation is, in this instance, similar to increased fitness. It was shown here that hybrid flies, with their intermediate sexual behaviour, do not mate more often in behaviourally diverse environments or milieus.

6. SEXUAL EFFICIENCY IN ADVANCED HYBRID POPULATIONS

6.1 Introduction

In the previous chapters I have outlined details of the taxonomy, biogeography, reproduction and sexual behaviour of Drosophila nasuta and D. kepulauana. In Chapter 5 the sexual behaviour of F_1 hybrids was described and mating efficiency was quantified. F_1 hybrids are intermediate between the parental species and uniform, and F_2 hybrids are behaviourally diverse. Most importantly these early-generation hybrids were not coadapted between the sexes (see sections 4.8 and 5.3.3) nor did they mate efficiently with either of the parental species. This conclusion is based largely on the relatively greater efficiency of non-hybrid sexual interaction and on the failure of F_1 hybrid females to respond to signals with an ibi distribution characteristic of F_1 hybrid males (section 4.8).

The survival of a hybrid population over many generations is evidence in itself of the ability of at least some individuals to achieve copulation and fertilization. In a bottle population even a single female can produce 'sufficient' progeny without there being any noticeable decline in adult numbers (section 4.2). Theoretically, flies in such a population could continue in this way unless indiviuduals with higher reproductive performance (i.e. greater fitness) arise. The 49 hybrid populations (Tables 6 and 7), under study here, showed no signs of decline or reduced fecundity throughout the 400 - 600 days that they were maintained. Of great importance then is demonstrating that there is actually an increase, from F_2 onwards, in the relative number of individuals sharing coadapted mating behaviour. Behavioural diversity and a low probability of being sexually coadapted is characteristic of early-generation hybrid flies but is it also characteristic of advanced generation hybrids? This is the crux of the present study: an investigation to see if there is restoration of coadapted character states or systems after their extensive disruption by hybridization. Does an advanced hybrid population share a common sexual recognition system?

It has often been observed that phenotypic characters when released from artificial selective pressure revert to a wild-type condition (Anderson 1949). This suggests the action of an underlying homeostatic mechanism operating either intrinsically at the genic level (Reanney et al. 1983) or interactively at the organismic level (Anderson 1949; Paterson 1985, 1986). The reversion to wild-type plumage among progeny of free-ranging, exotic pigeon breeds is a similar phenomenon. Apart from the study of Wallace et al. (1983) on D. pseudoobscura, D. persimilis and their hybrids, the present work is unique in testing homeostasis in reproductive behaviour after its radical disruption.

A working hypothesis is that if the behavioural heterogeneity in early F_2 hybrids is associated with varying abilities to achieve fertilization (variable mating efficiency) then advanced hybrids should be less variable and should achieve fertilization more efficiently (efficiency is defined below). This could be assessed by measuring the variability among many individuals of a single hybrid population over tens or scores of generations. The variability of F_2 individuals would be expected to be maximal while in parentals it would be expected to be minimal. Over a number of generations variability would be expected to decline and efficiency to increase towards parental values.

Since no software was available for computer analysis of TRACE data, it was not possible to score individuals in all populations for a change of variability through time. However, reproductive homeostasis i.e. the restoration of a wild-type mating system can be shown by comparing the courtship efficiency of hybrid and wild-type parental males. And, in addition, the efficiency of advanced hybrids can be compared to F_1 and F_2 hybrids of the same population.

The word 'efficiency' here refers to something better, quicker or less energetic. Dunbar (1982) in a discussion of adaptation, fitness and evolution places the term in the following context: 'Adaptation refers to some "problem set by nature" and entails the notion of a solution that permits the organism to overcome that problem efficiently ... The

criterion term "efficient" here conveys the notion of "design for a purpose" which I take to mean that a solution can be shown to be the most effective in terms of first principles. A character is said to be "better adapted" than any of its alternatives (qua competitors) when it permits its possessor to achieve the immediate objective set by that problem more rapidly, or at less cost energetically, or with greater frequency, or by whatever criterion is most appropriate to that problem.' Thus the efficiency of the courtship mechanism can be understood in terms of the frequency of successful accomplishments - of successful copulations, that being the purpose of the courtship mechanism. 'Rapid and efficient mating is an important component of fitness in Drosophila because it determines which individuals will transmit genes to the succeeding generation' (Connolly et al. 1974).

Efficiency is relative and it is comparable only in given contexts. The efficiency of hybrid courtship is not expected to be the same among different species. It should be emphasized that all judgements must be in relation to a specified context. For example, a nasuta male is efficient among nasuta females but among kepulauana females the same courtship would be highly inefficient. However, an index can be calculated (see below) which rates efficiency based on the effectiveness of courtship in two different but standard contexts.

The following study is the determination of the mating efficiency of advanced hybrid males. The standards to which they are measured are nasuta and kepulauana females. Hybrid mating efficiency is then compared to non-hybrid efficiency. The 'efficiency index' rates courtship from zero to unity. When males are determined to have an index of 0.00 they court with the efficiency of nasuta males achieving as many mates as do nasuta males in the two given contexts. When the index is 1.00 the males have the efficiency of kepulauana males.

Copulations may occur with unusually high frequency in a closed space because 'reluctant' females are unable to move away from males. The following trials are conducted under open conditions and it is assumed that only those copulations which involve fully cooperative females occur.

6.2 The Open-Cage Method

The 'Open-Cage method' is the name given to a technique designed to determine how many matings occur in 320 min when 60 males are placed with 50 females in a large ('open') space - the open-cage. This is quite distinct from calculating **Tq** and **Tsex** for individual males, it is also much easier. Advanced hybrid males from most of the 53 hybrid populations listed in Tables 6 and 7 (pp. 56, 57) were placed with either nasuta or kepulauana females (or sibling females) in more than 140 separate trials. Control trials involved the four possible combinations of males and females of the parental species.

The 'open-cages' were 11 l plastic buckets tightly covered with fine gauze. They are much more spacious than standard observation chambers (p. 45). In earlier behavioural studies (Chapters 4 and 5) the holding chamber or arena (Fig. 7) held flies at densities of 1.05 flies/cm^3 (30 flies in the arena) while the density of flies in the present apparatus is 0.01 flies/cm^3 (i.e. 110 in 11 l) and this density diminishes as mating pairs are removed. A 10 cm diameter hole in the side of the bucket, with a sleeve attached, allowed insertion and removal of flies. Squashed bananas covered with moistened absorbent paper lined the bottoms providing a humid atmosphere with a natural food resource.

The flies were kept on a 12 h light:12 h dark regimen for two generations before experiments. If flies are disturbed (e.g., by aspiration) during their sexually active period at the beginning of a light-phase (section 4.3, Wright 1977a), crowding and forced copulations occur and tests of sexual coadaptedness are spoiled. The problem of aberrant behaviour was overcome in these studies by introducing flies into the 'cage' well before scoring started. Five to six day-old virgin females were placed in buckets c. 15 h before a trial. Males (older than six days) were lightly etherized (for sexing) and placed in the buckets just after 'lights-out' during the 'night' before a trial. Thus, males and females were together in darkness in the cages for 10 to 12 h before trials began. Drosophila nasuta and D. kepulauana are sexually incompetent

during darkness (section 4.3) so mating did not occur before the start. During the 'night' before a trial, males recovered from the effects of etherization; feeding was possible. The flies were settled when the assays began each 'morning'.

Scoring commenced as soon as lights came on and ended after 320 min. Other experiments have indicated that most copulations occur in the first hour of a 12 h light phase (section 4.3) and only rarely occur after 240 min into the light phase (results not presented). To prevent the possibility of single males copulating with two or more females during a trial (this was shown to occur in other trials, section 4.9, Table 14, p. 93), mating pairs were removed. Long glass aspirators, inserted through the sleeve and hole in the buckets' sides, were used for this purpose. The time of removal was noted for each mating pair; temporal mating patterns are presented as frequency histograms based upon the times of removal of mating pairs. The 44 control experiments and c. 140 test experiments were run over three months at the end of 1985. A maximum of nine buckets (i.e. nine tests) could be monitored simultaneously during a 'morning'. (Note that scans for mating pairs in each bucket were necessary only once every c. 10 min since a mating lasts c. 20 min (section 4.6). Thus it was possible to scan buckets one at a time spending about 1 min on each and to be certain that no mating was missed). Buckets were set up side-by-side in an insectary with controlled temperature 23°+/-2.0°C and subdued lighting.

The Efficiency Index is derived from a comparison of tests and controls using Principal Component Analysis. For example, 60 males from a hybrid population may copulate with nearly all (e.g., 45/50) females of one species (say nasuta) during a trial. Then, in another trial, another 60 males from the same hybrid population may score few (e.g., 3/50) copulations with 50 females of the other species (kepulauana) under the same conditions. These two hypothetical scores, 45 and 3, are compared simultaneously with the results obtained when nasuta or kepulauana males are given the same two sets of females under the same conditions i.e. they are compared to a series of composite control scores.

The control scores are listed in Tables 1 and 2 in Appendix B and summarized in Table 21 (p. 157). In the first table of Appendiix B are listed the number of matings observed when 60 nasuta males are placed with 50 kepulauana females or 50 nasuta females; in the second table (App. B) are listed the results for kepulauana males. One of the results of a kepulauana x nasuta test is linked at random to one of the results of a nasuta x nasuta test. The same is done for trials involving kepulauana males. A composite control score is generated in this way. The entries in Tables 1 and 2 (App. B) are listed in the order in which they were randomly linked; each composite score (i.e. each randomly linked combination) is given a number (801-822). These composites are then compared one at a time with all other test composites. There were 12 replicates of tests involving nasuta males and 9 involving kepulauana males; but one of the latter is excluded (see below).

The analysis is a logical outcome of the combined confidence intervals of the nasuta and kepulauana controls. When confidence interval domains overlap at the 0.05 probability level the score is treated as being not significantly different and is rated -1. If there is no overlap in the 0.05 confidence interval domains then the rating is +1. The Efficiency Index is calculated from sums of significance ratings using the formula:

$$\textbf{Efficiency Index} = (20 + n - k) / 40$$

where n is the sum of significance ratings when the test score composite is compared to control composites 801 to 813 and k is the sum when the test score composite is compared to controls 814 to 822 (test 821 excluded). When the control results are variables in this formula the Index is 0.00 for nasuta and 1.00 for kepulauana. The hypothetical hybrid population (above) with the scores 45 matings with nasuta females and 3 with kepulauana females, has an Index of 0.00 i.e. nasuta-like at the 0.05 probability level.

In Tables 3 and 4 (App. B) the outcomes of such

comparisons involving all hybrid populations are given. In the body of these tables '-1' indicates no significant difference ($P<0.05$) and '1' significant difference ($P<0.05$) between tests (left entries) and control series (top row: 801 - 822). These 'ones' should not be confused with the Efficiency Index scores (listed under EFF. INDEX). The equation given above is used to determine the position of a population on the Efficiency Index scale. Figure 36 (p. 160) summarizes these indices for all hybrid populations analysed. Efficiency Indices and details of the origin and age of hybrid populations are also given in Tables 6 and 7 (pp. 56, 57).

If a hybrid population has an Efficiency Index value between zero and unity it may have a courtship which is relatively inefficient with both parentals. Alternatively it may indicate that males have super-efficient courtship. The latter produces an index between zero and unity. However, data presented below rules out this possibility. The populations with intermediate indices are discussed in detail.

As well as the above assay of hybrid male courtship with non-hybrid females (category 1, including results with F_1 males) some other tests were done. These fall into six remaining categories which are discussed separately. (2) Hybrid males have been tested with sib-females. (3) Non-hybrid males have been tested with hybrid females. (4) The males from four populations (numbers 27 to 30, Table 6, p. 56) which were started with equal proportions of non-hybrid nasuta and kepulauana males and females, were tested. (5) The sex ratio was varied to test the effect of this in the experiments. (6) The effect of offering females both nasuta and kepulauana males was briefly examined. And (7) the efficiency of D. kohkoa, a closely related species and some distantly allopatric but conspecific strains of nasuta, were examined with the Open-Cage method. The hybrid-with-hybrid tests are limited because of the practical difficulties of expanding populations to yield sufficient numbers of virgin females at any one time.

Because hybrid populations were started at different times the age of populations is a variable; it should be noted that there is considerable variability. The oldest population

is number 01: 799-813 days; the youngest populations are number 26 (136-161 days) and numbers 34 and 38 (175-201 days); the majority of the remainder are either c. 285-320 days or c. 445-470 days. Tables 6 and 7 give the age of all the populations at the time when the hybrid males were tested with kepulauana females (AGE') or nasuta females (AGE"). It has not been possible to give a precise filial number to the populations because the culturing procedure allowed overlapping generations. All that can be given is the maximum filial or generation number, the age in days divided by the shortest generation time i.e. 13 days (see section 4.2) and the minimum filial number, the age divided by 25, a very approximate estimate of the maximum generation time.

In section 4.3 it was found that most (80%) nas-with-nas copulations occur within the first 60 min after simulated dawn. The results of experiments with kepulauana (data presented below) show that their daily mating pattern is different, being more spread out. Thus if advanced hybrid males evolve a courtship which is as efficient as that of nasuta, for example, it is predicted that with nasuta females matings will have a temporal frequency distribution matching nas-with-nas. Conversely, hybrids which mate efficiently with kepulauana and not with nasuta would be expected to have patterns similar to the copulation frequency distribution of kep-with-kep.

6.3 Results

Control Results: The results of the kepulauana and nasuta control tests are summarized in Table 21 (p. 157, see also Table 16, p. 96). It was found that when 60 nasuta males were placed with 50 kepulauana females in the Open-Cage for 320 min an average of only 2.3 (4.6%) females mated (sd = 1.93, V = 3.44, 13 replicate trials). In contrast, when nasuta males were placed with nasuta females, under the same conditions, an average of 46.3 (92.6%) females mated (sd = 4.07, V = 15.3, 13 replicates, Appendix B). When 60 kepulauana males were placed

Table 21. Percentage of females mated in Open-Cage trials. Ratio of males to females is 60:50 each time. 'nas' and 'albom' = *Drosophila nasuta*; kep = *D. kepulauana*. Details of stocks given in Table 3. Results of 44 separate Open-Cage trials presented; results in each row are independent.

MALES	% FEMALES-MATED		MALES	% FEMALES-MATED	
	nas-1	kep-1		nas-1	kep-1
nas-1	100.0	4.3	kep-1	62.0	52.0
nas-1	96.0	14.0	kep-1	34.0	60.0
nas-1	90.0	6.0	kep-1	54.0	62.0
nas-1	100.0	0.0	kep-1	18.0	50.0
nas-1	96.0	4.0	kep-1	10.0	40.0
nas-1	68.0	4.0	kep-1	12.0	32.0
nas-1	92.0	2.0	kep-1	36.0	56.0
nas-1	90.0	2.0	kep-2	18.0	46.0
nas-1	-	10.0	kep-3	44.0	48.0
nas-1	-	0.0			
nas-1	-	6.0	**means:**	32.0	49.6
nas-1	-	4.0			
nas-3	98.0	-			
nas-18	95.7	-			
albom-1	92.0	4.0			
albom-1	92.3	-			
albom-1	93.8	-			
means:	92.6	4.6			

with 50 *kepulauana* females an average of 24.8 (49.6%) females mated (sd = 4.76, V = 20.2, 9 replicate trials). When they were placed with 50 *nasuta* females an average of 16 (32.0%) females mated (sd = 9.39, V = 78, 9 replicates).

Figure 35 (p. 158) shows the temporal mating pattern, over 320 min, for the four pair-wise combinations of *nasuta* and *kepulauana*. The frequency histogram for nas-with-nas has already been indicated in studies of sexual activity rhythms (section 4.3), compare Figures 9 (p. 69) and 35. Each column in the histogram represents 'number of copulations observed in

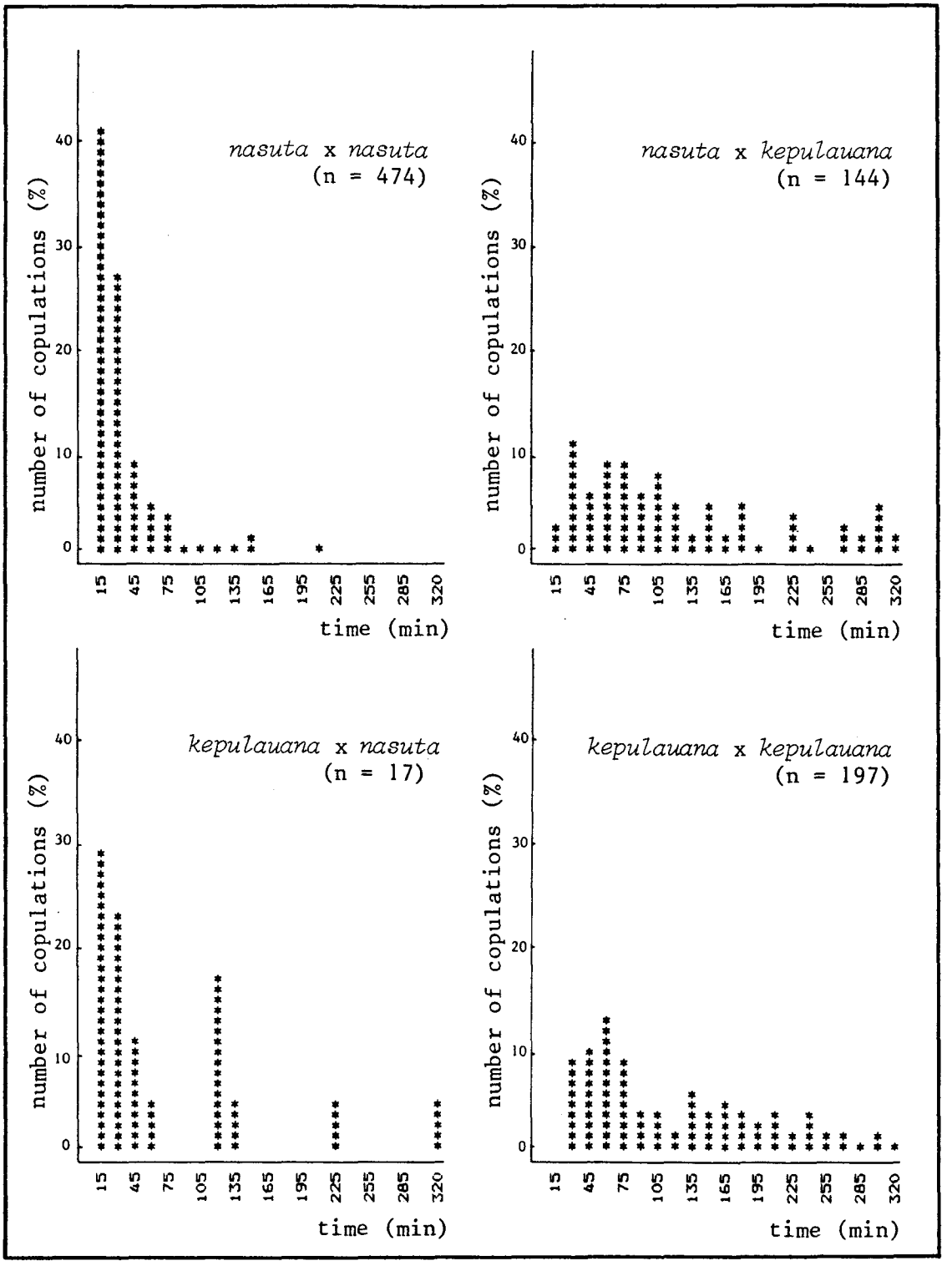


Figure 35. Histograms depicting temporal mating patterns of D. nasuta, D. kepulauana and reciprocal combinations during Open-Cage trials; n, total number of copulations observed; crosses in the order: 'female x male'.

a 15 min interval' as a percentage of the total number (n), during an Open-Cage trial. Thus, 41% of the nas-with-nas matings were within the first 15 min and 93% occurred before 75 min. In contrast, no flies in the kep-with-kep trials mate within the first 15 min and only 41% of all copulations observed occurred within the first 75 min.

The trends described above are consistent with the hypothesis that it is the male that determines the nature of the pattern. When the females are nasuta and the males kepulauana (Fig. 35) a pattern similar to the kep-with-kep result is obtained. Similarly the reciprocal combination, kepulauana females x nasuta males, yields a mating pattern approximately similar to the nas-with-nas result. (With respect to the kep-with-nas results, the histogram may be distorted due to the low total number of observations; however, the results in the four histograms for the first 15 min are unequivocal). It may be concluded that a nasuta characteristic is that, under the present conditions, most copulations occur within the first 75 min of light, during a 320 min trial. In contrast, kepulauana males reach a peak of sexual activity at the 60 min mark which diminishes only gradually (cf. nasuta) throughout the remaining 260 min of a trial. Indeed it may be that for kepulauana the 320 period is too short to include all matings affected during a day (cf. nasuta). This may explain why so few kep-with-kep matings were observed in total compared to nasuta (Table 21); this has not been tested. The mating frequency patterns, involving hybrid males from different populations, are compared to these non-hybrid 'standards' (see below).

With respect to the number of copulations there is no ambiguity in the two control series, except test 821. It is assumed that the very low kep-with-kep score of test 821 (Table 2, Appendix B) is due to a methodological artifact. Perhaps a male was present among the kepulauana females while they were being 'aged' before the trial. Test 821 is rejected from further analyses. This adjustment is permissible because an omission of this type is statistically acceptable at the 0.05 probability level (Rouault, pers.comm.).

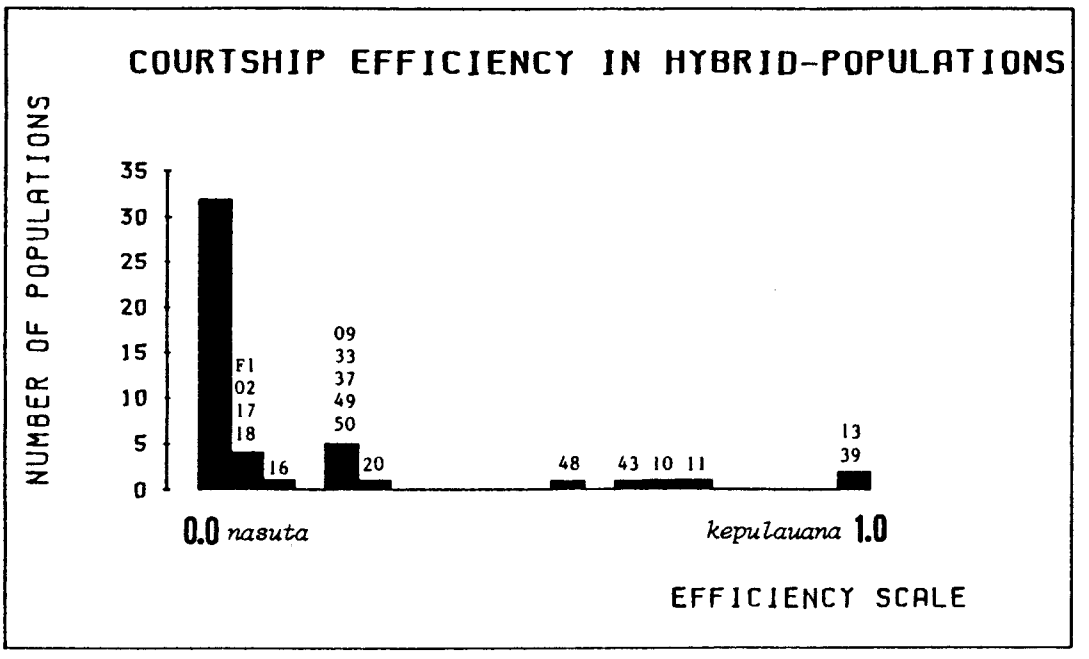


Figure 36. The courtship efficiency in 48 hybrid populations after many generations (mating efficiency scale).

(1) Non-hybrid females with hybrid males: Results are given in Tables 3 and 4 in Appendix B and summarized in Figures 36 and 37 (p. 161). Supplementary data about the frequency of mating during the 320 min trials, are presented in a series of simple histograms (Figs 35, 38-40).

Figure 36 summarizes the range of Efficiency Index scores for the 49 hybrid populations studied. Population numbers are given above each bar, except the bar at left which comprises all remaining hybrid populations. On the right of Figure 36 populations 39 and 13 have males as efficient as kepulauana, populations on the left of the scale have males as efficient as nasuta. Populations with intermediate efficiency indices differed significantly ($P<0.05$) from composite control scores and were neither kepulauana-like nor nasuta-like in their mating efficiency.

The overall trend towards the nasuta SMRS in advanced nasuta x kepulauana hybrids is also reflected in the principal component plot (Fig. 37); there is a 0.95 probability that each ellipse contains all observations. There is good separation of the controls with respect to both axes.

Figure 36 shows that of the 48 advanced hybrid populations, 32 (67%) court with the efficiency of nasuta. This is perhaps the single most important finding of the whole study. The males of these 32 populations copulate freely with nasuta females and seldom with kepulauana females. The results for all such populations with an Index = 0.00 (nasuta-like) are listed together in Table 4 (Appendix B). Results for the other hybrid populations are listed in Table 5 (Appendix B). At the right end of the Efficiency Scale (Fig. 36), advanced hybrid males of only two of the 48 hybrid populations (4%) court with the efficiency of kepulauana. The males of these two populations (13 and 39) score a relatively high number of copulations with kepulauana females and a low number of copulations with nasuta females. These males are as efficient as kepulauana males.

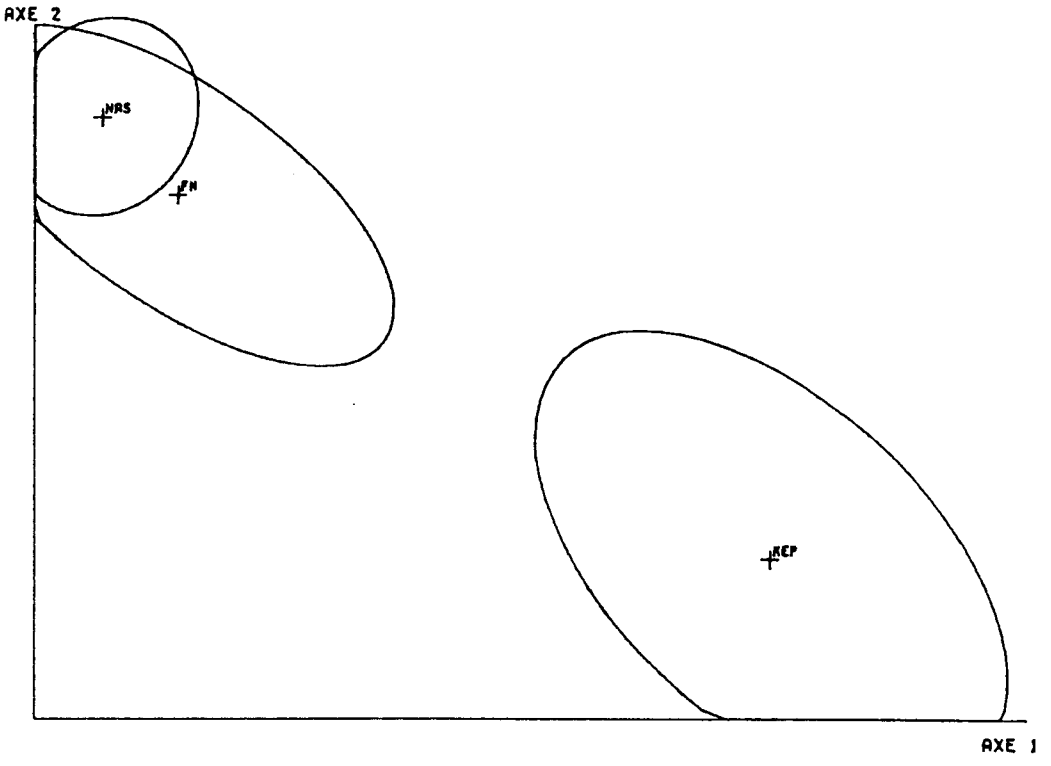


Figure 37. Projection of 48 nasuta x kepulauana hybrid populations and controls after a principle component analysis of their ability to mate with kepulauana females (abcissa) or nasuta females (ordinate). KEP - Drosophila kepulauana; NAS - D. nasuta; FN - advanced hybrids.

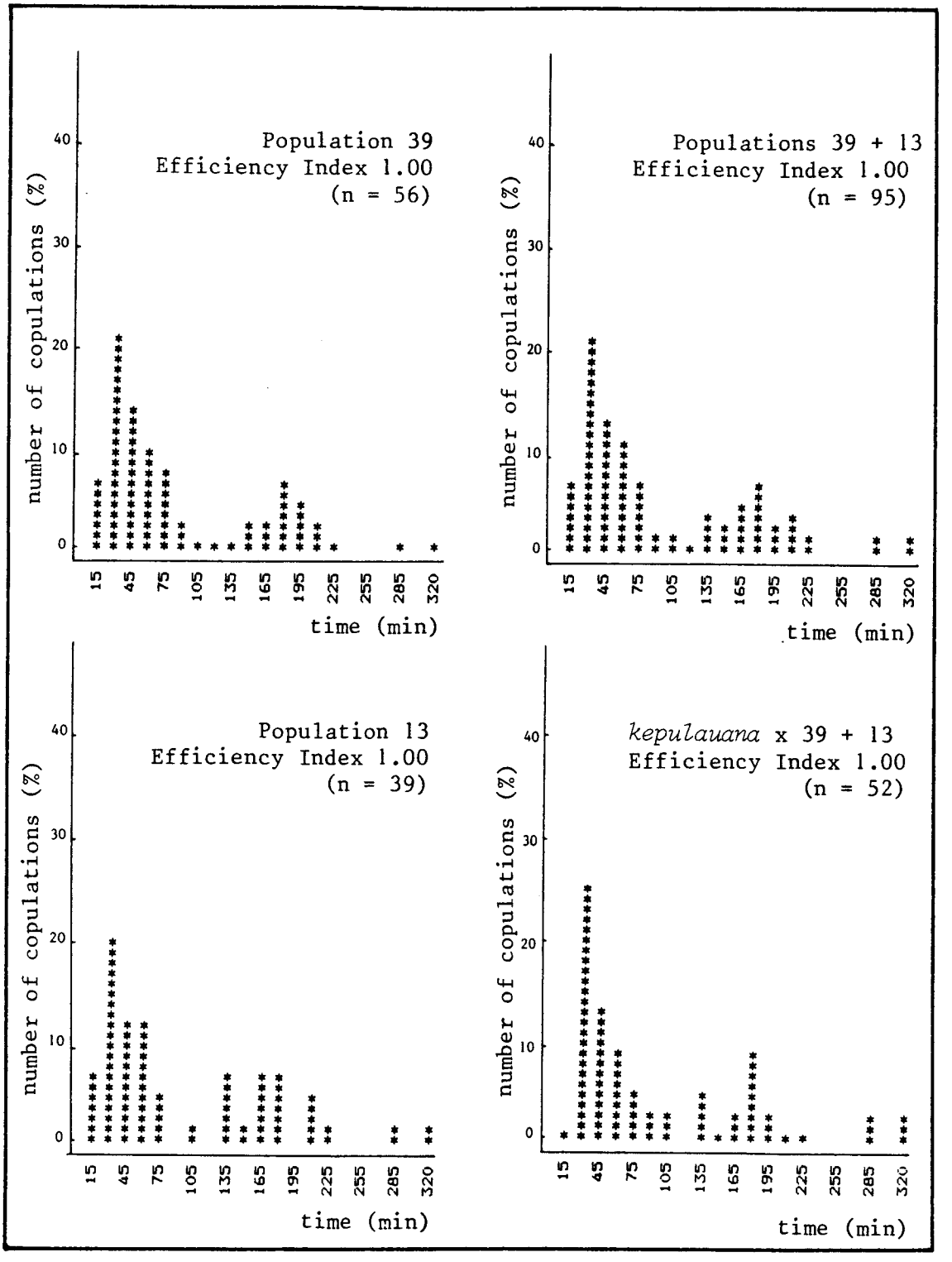


Figure 38. Histograms depicting temporal mating patterns of males from the two hybrid populations 39 and 13 which have the mating efficiency of kepulauana. (See Fig. 35 for key.)

Figure 38 shows the temporal mating pattern of the two advanced hybrid populations (39 and 13) found to be kepulauana-like (Index = 1.00). Results with nas and kep females were pooled to produce the histogram labelled 'Population 39' (the assumption that the type of female does not alter the pattern, is made here). The 'Populations 39 + 13' histogram is the pooled data for males of these populations tested with both nas and kep females; the 'Population 13' histogram summarizes data for all trials involving males from population 13; the last histogram shows data from all trials involving only kepulauana females and males from both hybrid populations. In each histogram the temporal patterns are similar to the kep-with-kep mating frequency distribution (Fig. 35). There is a very low number of matings observed in the first 15 min and there is a relatively high percentage (c. 20%) of all matings occurring between 165 and 225 min after 'lights on'. This should be compared to the kep-with-kep value for the same interval (i.e. 18%, Fig. 35) and the very low nas-with-nas value of 1% (Fig. 35). These two hybrid populations therefore have a temporal mating pattern similar to kepulauana and unlike nasuta as well as having an Efficiency Index of 1.00.

Results of the remaining 14 populations which have an Efficiency Index greater than 0.00 but less than 1.00 are listed in Table 3 of Appendix B (see also Fig. 36). Males from these hybrid populations have relatively inefficient courtship in terms of the number of matings they achieve with parental species females. Among these there are three groups: (1) populations 02, 17, 18, 16 and F_1 hybrids which have an Efficiency Index of 0.05 or 0.10; (2) populations 09, 33, 37, 49 and 50 with indices of 0.20 or 0.25; and (3) populations 48, 43, 10 and 11 with indices between 0.55 and 0.75 (Tables 6 and 7, p. 56; Fig. 36, p. 160; Table 3, Appendix B). The temporal mating pattern of only one of these hybrid populations, 43 (Efficiency Index 0.65), is given (Fig. 39, p. 167). One of the histograms in Figure 39 shows the frequency distribution of mating when siblings from population 43 are tested together using the Open-Cage method. A pattern like kep-with-

kep (Fig. 35) is observed; the distribution is quite unlike nas-with-nas (Fig. 35) because there is no clear peak in the first 15 min and there is an attenuated distribution of values extending to about 225 min.

Of the 48 hybrid populations analysed, 25 were started with the F_1 progeny of a cross between the same two iso-female strains (i.e. nas-1 x kep-1, yielding populations numbered 01 to 26, Table 6). The remainder are the progeny of crosses between various iso-female strains (i.e. nas-1 to nas-17 females x kep-1 and kep-2 males yielding populations numbered 31 to 53, Table 7). There are, therefore, approximately equal (25 vs 23) numbers of populations in these two categories. All populations possess the Y-chromosome of kepulauana. In Figure 36 it can be seen that the distribution of efficiency ratings is independent of the type of strains involved in the initial hybridization. One of the nas-1 x kep-1 populations has an index of 1.00 (population 13) and similarly one from the other series (population 39, nas-13 x kep-1) also has an index of 1.00. In the group which have an intermediate Efficiency Index (0.55 to 0.75), two are from the nas-1 x kep-1 series and two are from the other series. This pattern continues to the left of the scale in the figure as well. Nor is there a trend on the scale (Fig. 36) which is attributable to the age of a population. The oldest (number 01) and youngest (number 26) populations both have males which court like nasuta, they form part of the large bar on the left of the scale in Figure 36. Among the populations to the right of the efficiency scale and in an intermediate position there are approximately two age-groups. Population numbers 39, 43 and 48 are about 450 days old (c. F_{25-30}) and populations 10, 11 and 13 are about 290 days old (c. F_{16-20}).

Of great interest, and in apparent contradiction with the results presented in the previous chapter, is the fact that the F_1 males mate readily with nasuta females. In five tests with nasuta females the percentage copulations were 80%, 88%, 90%, 92% and 92%. In contrast kepulauana males mate on average with only 32% of available nasuta females. With kepulauana females one group of 60 F_1 males scored 15 matings (more than

did any other male type except, of course, kepulauana males) and in another test with kepulauana females 60 F_1 males scored only 2 copulations. The Efficiency Index for F_1 males is therefore very low, close to nasuta. Further tests of F_1 males with kepulauana females are needed since there is a large variation in the above results. No additional tests were carried out because several generations are required to expand cultures so that they produce sufficient numbers of 'same-age' flies for testing (see below). Groups of 60 males are not scored twice with the Open-Cage technique so the two Efficiency Indices (Table 3, Appendix B, F1.a and F1.b) which have been calculated for F_1 males are the results of four tests involving 240 F_1 hybrids.

When nasuta females are courted by both nasuta and F_1 hybrid males ('choice' situation) they seldom respond positively to hybrids; they move away from them more often than from conspecifics (section 5.3.3). The Open-Cage situation offers 'no-choice' to females and this is often associated with high rates of 'outbreeding' or heterotypic mating (p. 95; Spieth 1966).

(2) Hybrid males tested with their sib-females. Several months are required to expand cultures to a size which will yield large numbers of virgin females daily, as required. If nine tests are done daily 450+ virgin females are required daily. The kep-1 stock was expanded first and then the nas-1 stock (thus the difference in ages - AGE' and AGE" in Tables 6 and 7 - when hybrid males were assayed with virgin kepulauana or nasuta females). There are only limited data concerning the coadaptedness between hybrids as measured with the Open-Cage technique because of these culturing difficulties.

Sufficient virgin females were, however, obtained from five populations. Two (populations 43 and 48) were chosen because of their intermediate Efficiency Indices. Males from the oldest population (01) were tested with their sib-females. And two young populations (numbers 25 and 26, 194 and 149 days respectively, c. F_{9-12}) were assayed. Replication was not possible. The control results are summarized in Table 21.

Results of hybrid males with their sib-females and results of assays of F_1 hybrids are given in Table 22.

Table 22. Percentage of females mated in Open-Cage trials. Hybrid males placed with nasuta or kepulauana females (two left columns) or with sib-females. Ratio of males to females is 60:50. For population code numbers (left) refer to Tables 6 and 7. Bracketed entries are replicates.

MALES	% FEMALES-MATED							INDEX
	nas-1	kep-1	01	25	26	43	48	
nas-1	92.6	4.6	96.0	92.0 (70.0)	-	-	-	0.00
kep-1	32.0	49.6	46.0	42.0	46.0	-	-	1.00
01	94.0 (100.0)	2.0	96.0 (92.0)	-	-	-	-	0.00
25	96.0	2.0	-	90.0 (84.0)	-	-	-	0.00
26	92.0	8.0	-	-	82.5 (92.0)	-	-	0.00
43	48.0	2.0	-	-	-	41.9 (38.7) (51.6)	-	0.65
48	60.0	36.0 (36.0)	-	-	-	-	12.0	0.55
F_1.a	80.0	4.0	-	-	-	-	-	0.00
F_1.b	92.0	30.0	-	-	-	-	-	0.05
F_1	(88.0)							
F_1	(92.0)							
F_1	(90.0)							

After about 50 generations the sexual behaviour of both males and females in population 01 were found to be the same as D. nasuta. The temporal mating pattern when siblings were tested together (Fig. 39, p. 167) was very close indeed to the nas-with-nas pattern (Fig. 35). With respect to the actual number of matings, the males achieve many copulations (94 and

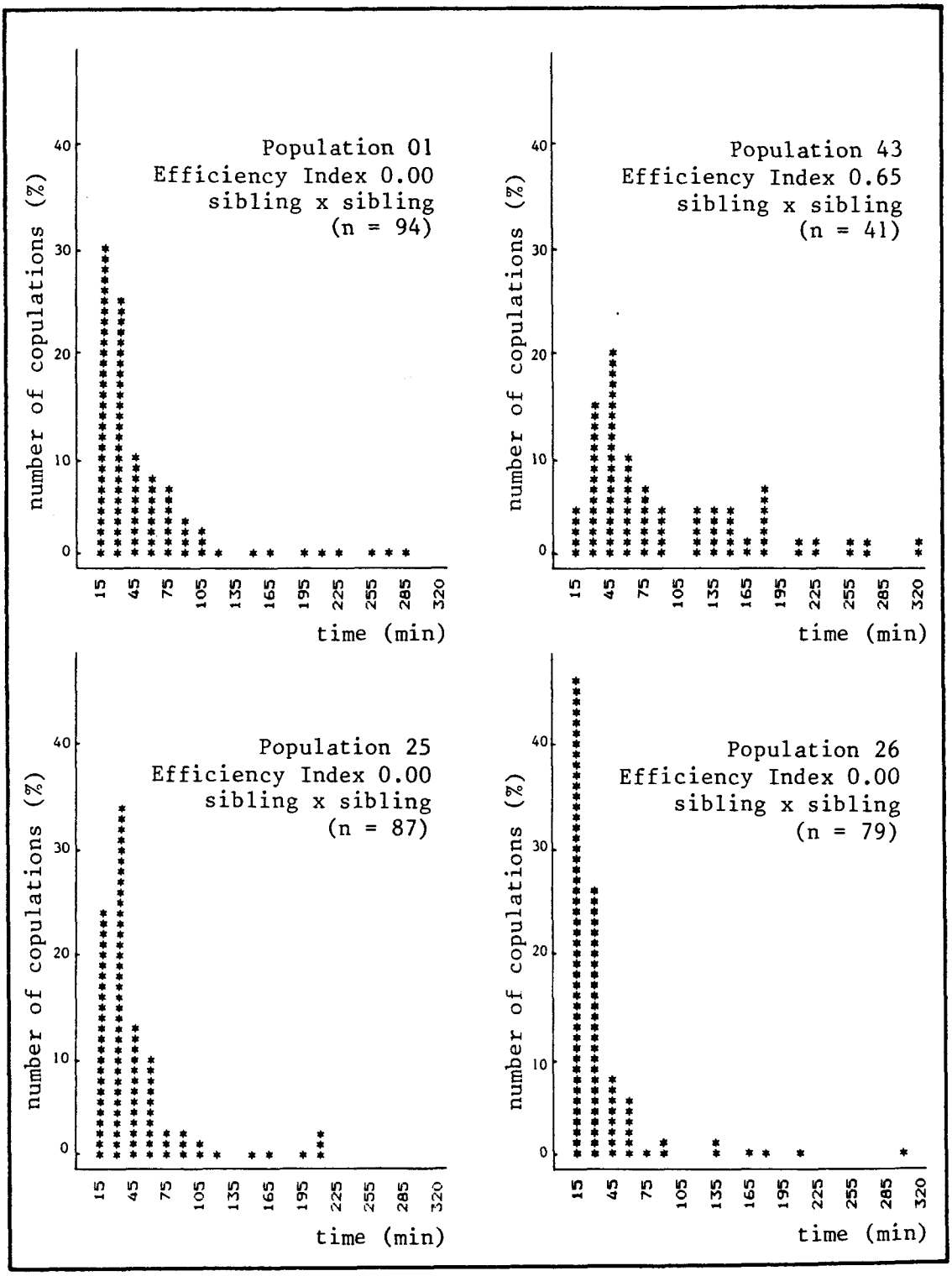


Figure 39. Histogram depicting temporal mating patterns when advanced hybrid males are tested with their sibling females. (See Fig. 36 for key to abbreviations).

100%) with nasuta females and they mate as effectively with their sib-females (96 and 92%). They are inefficient with kepulauana; like nasuta, 60 males succeed in mating with only one (i.e. 2%) out of an available 50 kepulauana females in 320 minutes. The 01 females respond poorly to the courtship of kepulauana males and only 46% mate. This is similar to the behaviour of nasuta females. Males and females in population 01 are as sexually coadapted as nasuta.

Populations 25 and 26 are only about 10 generations old and yet their sexual behaviour is very like that of nasuta. Of interest, however, is the slightly reduced efficiency of the males with their sib-females (only 82-92% of possible matings occurred). Under the present conditions the sexual signalling is, however, more efficient than that between kepulauana males and females: kepulauana males never mated with more than 62% of the available kepulauana females.

The temporal mating patterns of males from 25 and 26 are presented in Figure 39 (p. 167). Only 24% of all matings by hybrid 25 males occur in the first 15 min (cf. 41% nas-with-nas), this is unlike the nasuta pattern. However, since there is no gradual attenuation the overall impression is that the distributions match nasuta more closely than kepulauana.

Populations 43 and 48 are older than 25 generations. Population 43 has a sexual efficiency very unlike nasuta; 60 males mated with only 24 out of 50 (48%) available nasuta females; this is as inefficient as kepulauana males. But at the same time the hybrid males have a sexual behaviour which is extremely inefficient with kepulauana females. With both nasuta and kepulauana females the mating scores are minimal. With sibling females a score which is comparable to kep-with-kep was obtained; the average of the three percentages presented in Table 22 (p. 166) is 44.1% while kep-with-kep averages 49.6%.

The other population which has an intermediate Efficiency Index and from which enough virgin females could be retrieved, population number 48, presents a similar picture. Except that the score with sib-females (12%) is so low one might wonder how the population could have survived (but see p. 149). No

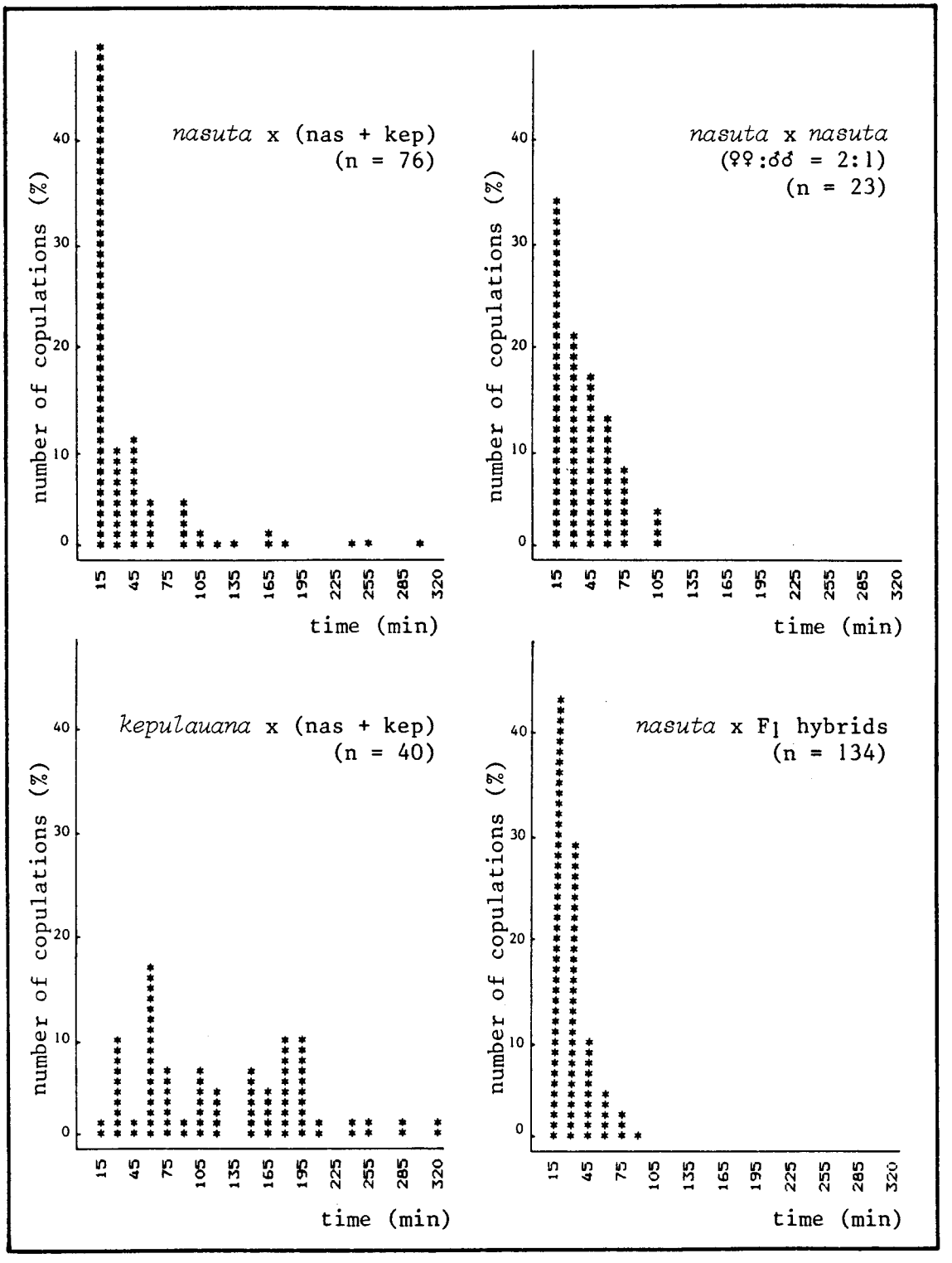


Figure 40. Histograms depicting temporal mating patterns of nasuta, kepulauana and F_1 hybrid males. (See Fig. 36 for key to abbreviations; 'nas + kep', 30 nasuta and 30 kepulauana males).

other within-population score is as low. Of 50 available sib-females 60 males mated with only six (12%); the closest lowest score is a kep-with-kep value of 16 matings (32.0%). Because data from replicate trials are unavailable it is possible that this is spurious. (Note: these scores represent the outcome after 320 minutes. Higher scores would result after more time. Furthermore, a 12% fertility among females is ample to maintain large populations.

First generation hybrid males have not been tested with their sib females in the Open-Cages. The overall impression is that F_1 males have a courtship efficiency which is truly intermediate and which achieves less matings than either nas-with-nas or kep-with-kep but more matings than either kep-with-nas or nas-with-kep.

In Figure 40 the temporal mating pattern of nasuta females with F_1 hybrid males is given. The results from several trials are presented; of 134 copulations 43% occurred within the first 15 min. After which there was a sharp decline, after 75 min few (1%) flies mated. This pattern is almost identical to the nas-with-nas graph in Figure 35. Since several advanced generation hybrid populations have high mating frequencies after 75 min it is possible to argue that hidden within the F_1 phenotype is the genetic potential to be sexually active at later times in the day but that this behaviour has low penetrance in generally heterozygous hybrids.

(3) Non-hybrid males with hybrid females. Females from only two hybrid populations have been tested with nasuta and kepulauana males - the very advanced population number 01, and the young population 25. 46% of 01 females mate with kepulauana males, this score lies within the range of nas(fem)-with-kep scores and is nasuta-like. The nasuta males mate with nearly all 01 females which is also consistent with the conclusion that population 01 has fully recovered a nasuta mating system. The percentage scores (Table 22, p. 166) for population 25 indicates that these hybrid females mate more readily with nasuta males than they do with kepulauana males (92% and 70% versus 42% respectively).

Table 23. Percentage of females mated in Open-Cage trials - an analysis of variable sex ratio and variable male composition. Controls (represented here as mean scores) have the standard 60:50 sex ratio (males:females) and males are either all *nasuta* or all *kepulauana*.

MALES	% FEMALES-MATED		SEX RATIO
	nas-1	kep-1	male:female
nas-1	92.6	4.6	60:50
kep-1	32.0	49.6	60:50
nas-1	57.5	-	30:50
kep-1	2.3	-	30:50
(nas-1+kep-1)	78.0	40.0	(30+30):50
(nas-1+kep-1)	74.0	46.5	(30+30):50
D. kohkoa	-	0.0	60:50
D. kohkoa	0.0	-	60:50

(4) Populations started with equal proportions of non-hybrids. The four populations: 27 to 30 were started, not with hybrids but with equal proportions of non-hybrid *nasuta* and *kepulauana* flies. The initial ratio in each bottle-population was 15 *nasuta* males : 15 *nasuta* females : 15 *kepulauana* males : 15 *kepulauana* females. Flies were all the same age and virgins at the beginning. After one generation the number of flies per bottle was kept between 100 and 200 as was the case with all bottle populations. After 150 days (c. 8 to 10 generations) 60 males from each bottle were tested with *nasuta* in the Open-Cages. The results (Table 23): 74.4%, 78.4%, 85.0% and 76.3% are inconclusive. These values match very closely those obtained when 30 *nasuta* males and 30 *kepulauana* males are placed together in an Open-Cage trial with 50 *nasuta* females (see Table 23 below). In other words the data could result either from a dimorphic, non-interbreeding population with approximately equal proportions of non-hybrid *nasuta* and *kepulauana* flies or from a fully introgressed population with a signalling system maladapted to the *nasuta* sexual signalling system. It was possible however to review some film of the

male behaviour when the populations were about 60 days old. The interburst-interval (section 4.8) and the starting positions clearly demonstrate that hybridization had occurred.

(5) Sex ratio as a variable in Open-Cage tests. The ratio of 60 males to 50 females was chosen for the Open-Cage experimental test because in preliminary trials it was found that fewer males (e.g., 50) failed to mate 100% of the females in the 320 min. With the 60:50 ratio, nasuta males could sometimes mate all the available females. What is the effect of reducing the ratio of males to females?

Table 23 (p. 171) includes results of two trials in which the number of males was halved: 30 nasuta with 50 nasuta and 30 kepulauana with 50 nasuta. The controls are the scores when 60 males are introduced. If density plays no role it is reasonable tp predict that half as many matings occur. In the first trial this predicted score is 46.3% (i.e. 92.6/2). The observed is 57.5%. Changing the abundance of males apparently has an effect. It may alter the probability of encounter, the average number of males that court each female or the amount of general activity. The temporal mating pattern of nas-with-nas when the sex ratio is 5:3 (females:males) is given in Figure 40 (p. 169). The pattern, when compared to nas-with-nas in Figure 35 (p. 158), suggests that males take longer to achieve the same number of matings.

In the second trial (30 kepulauana : 50 nasuta females), reducing the number of kepulauana males has a drastic effect on the percentage of nasuta females copulated. The trend is in the opposite direction to the one observed in the first trial. The expected frequency of 30 kepulauana with 50 nasuta is 16% (32.0/2) but the observed is a mere 2.3%. Thus when nasuta females are surrounded by - and courted by - few kepulauana males, in a ratio of 3:5, they are less likely to mate with them than if the males are more abundant (at 6:5). If this trend is linear it may be that a ratio of 2:5 or 1:5 results in no hybridization between kepulauana males and nasuta females in the open cage. Time was not available to pursue this hypothesis.

(6) Open-Cage with 'female choice'. What is the result when 30 nasuta males plus 30 kepulauana males are placed firstly with nasuta or with kepulauana females? Table 23 (p. 171) gives the results of two such Open-Cage experiments (each replicated twice). Expected values are problematic because of the density dependent effects outlined above. There are two ways of deriving an expected value. One could combine the two '30:50' scores (i.e. 57.5 + 2.3 = 59.8) or one could combine half the scores yielded in the '60:50' trials (i.e. 92.6/2 + 32.0/2 = 62.3 or 4.44/2 + 49.56/2 = 27.0). However, both ways yield approximately the same result (e.g., 62.3 vs 59.8) with nasuta females.

It was predicted that in trials with 30 nasuta + 30 kepulauana males about 60% of the 50 available nasuta females would mate. An average of 76% mated. Similarly in the two replica trials with kepulauana females the prediction was that about 27% would mate. An average of 43.3% mated. In these trials females have higher receptivity when both types of male are present although they apparently continue to mate assortatively. The temporal mating patterns of trials involving either nasuta or kepulauana female 'choice' are given in Figure 40. The patterns are consistent with the hypothesis that only conspecific males are involved in the matings observed.

(7) The mating efficiency of other species. When males of Drosophila kohkoa (a cryptic species said to be closely related) are placed in an open cage with nasuta or kepulauana females they fail, completely, to mate (Table 23). Indeed no courtship was observed although observations were not continuous. The kohkoa males are apparently not at all responsive to the signals of either type of female, nor vice versa. Note that kohkoa has been reported to hybridize with nasuta and kepulauana (see Table 5, p. 53) but no doubt under confined and artificial conditions.

One last piece of evidence relevant to the evolution of mating systems within a single population comes from some experiments that were presented in part in section 4.9 (Table

Table 24. Statistical summary of tests of the null hypotheses: that F3 hybrids (hyb) are different to F7 hybrids with respect to their mating success with nasuta (nas) and kepulauana (kep). (*, P<0.05; n.s., not significant; see also Table 15).

mating type	theoretic	obs.	conf.interval	
F3 hyb x kep	5/10 (0.50)	2/4	0.147 - 0.853	n.s.
F3 hyb x nas	5/10 (0.50)	2/4	0.147 - 0.853	n.s.
F7 hyb x kep	5/10 (0.50)	0/5	0.000 - 0.459	*
F7 hyb x nas	5/10 (0.50)	5/5	0.541 - 1.000	*

14, p. 93). In the present chapter population 01 has been shown to have recovered the nasuta SMRS after 15+ generations. When this population was in its third generation, 01.F_3 males were analysed using TRACE. It was found that there was no significant (P<0.05) deviation from random mating; some males mated with kepulauana females, some with nasuta females and others with their sibs (Table 14). However, by the seventh filial 01.F_7 males were mating only with nasuta or sibling females; no longer were they mating at random. Table 24 is a statistical summary of the test of the hypothesis that F3 and F7 hybrids are different with respect to their mating success. It shows that there is a significant difference and by inspection it can be seen that this is because the F_7 males mate entirely with nasuta females and no longer with kepulauana. The recovery of the nasuta SMRS was apparently underway before the seventh generation.

6.4 Discussion

In this chapter I have shown that the mating behaviour in hybrid populations changes through time. Sexual behaviour was shown to be intermediate and inefficient among first generation hybrids and variable in the F_2 (Chapter 5). However, in this chapter I have shown that among most advanced hybrids the sexual behaviour has polarized. Furthermore it has become uniform, and, in most populations, efficient with a

parental species.

In the majority of these artificial hybrid swarms, the nasuta SMRS has been reconstituted by natural selection. A small fraction (2/48) have reverted to the kepulauana SMRS and the males of some 14 populations are still sexually inefficient perhaps trapped in this condition by the random extinction of components of one or other or both of the parental mate recognition systems.

It was found that early generation hybrid males were more successful with nasuta than with kepulauana females in the Open-Cages but it is known that they expend more energy in achieving syngamy (Chapter 5). In this observation may lie an explanation for the above polarization towards nasuta. Drosophila nasuta females respond to a broader range of signals than do kepulauana females and F_1, F_2 and F_3 hybrid males court at random. Thus the restoration of the nasuta SMRS, in most populations, would be explained if nasuta-like female hybrids had a reproductive advantage and are favoured by natural selection. I suggest that such females mate with males from both the large group of intermediate forms as well as males which are more nasuta-like, whereas kepulauana-like hybrid females respond only to the small proportion of kepulauana-like hybrid males in each early hybrid population. Consequently more populations have reverted to the nasuta form of SMRS than to the kepulauana form. The fact that some populations have a restored kepulauana SMRS suggests that stochastic processes have led to the random extinction of important components of the nasuta SMRS. The extinction of important behavioural components of both the nasuta and kepulauana SMRS's might explain the 29% of advanced hybrid populations which have intermediate Efficiency Indices after many generations.

Drosophila kepulauana females are responsive to a narrow range of male signals; this behaviour is disadvantageous in hybrid swarms and has been eliminated by natural selection.

Those who have confidence in the reinforcement model of speciation might interpret the divergent temporal mating patterns of nasuta and kepulauana as good evidence for repro-

ductive character displacement. They might even go so far as describing it as a reproductive isolating mechanism. If two closely related and potentially interbreeding species mate at different times it might be supposed natural selection favoured non-overlapping mating periods. However I have shown in section 4.3 that if males fail to mate in the 'morning' they become sexually active again in the 'afternoon' at a time when it appears that kepulauana females are receptive. Furthermore, I have clearly demonstrated that even though the nasuta and kepulauana males mate at different times of the day (Fig. 35), females do not appear to have a temporal preference.

7. DISCUSSION

Too much an animal to fear death.*

The chilling rationalization that one's own existence is temporary was a transcending event in the history of man's knowledge. Death is absolute and infallible and anyone who can foresee it fears it. What horror must have filled the mind of Early Man when he first acquired an ability to reason and was then able to envisage his own mortal fate. Is it any wonder then, that at the root of most, if not all, religion and primeval folk-lore we find a special parable of death followed by reincarnation or resurrection. Because of the ubiquity of such a tendency in the human psyche, even in widely isolated human populations, it seems likely that the existence of 'now-and-hereafter' superstitions is an ad hoc solution, arising as a comforting psychological remedy for perpetuating life, for avoiding death or for escaping self-obliteration. Thus, inevitably, man is predisposed to think about life in terms of how the living and extant solve 'the problem of survival' and avoid extinction. Scientists are no more immune from such deep-seated prejudices than the rest of mankind and many a scientist is surrogate to various, often incompatible, philosophies or metaphysics (Ghiselin 1974:7). Given the reach of these prejudices it is perhaps not surprising that a large body of contemporary biologists hold the belief that ad hoc mechanisms exist which act to conserve organic integrity by preventing panmixis; organic diversity is viewed as evidence of the reality and effectiveness of such mechanisms. In my opinion the existence in man of these convictions is the product of a deep-seated aversion to extinction.

Embracing Mayr and Dobzhansky in the sweep Ghiselin (1974:x, 30-31) has described this orthodoxy as vitalism. Of course, this appellation is generally repugnant to moderns and

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* From the execution scene in Jean-Pierre Melville's film 'L'Armée des Ombres'.

yet vitalistic notions persist as pervasive and beguiling undercurrents. Skolimowski (1974) contends that 'though God is denounced and declared non-existent, his shadow still haunts the biologist turned natural philosopher.' The alternative 'mechanistic' world view is that life is fortuitous, that no vital force produces or maintains it and that chance and natural selection alone have physically created it. The present diversity of life is an automatic consequence of physics, chemistry and the process of natural selection (Darwin 1858). 'Grasp the mechanism that produces change and all else follows' (Ghiselin 1974:7).

'We are dealing with life when we are forced to invoke natural selection to achieve a complete explanation of an observed system' for the principles of physics and chemistry alone are not enough (Williams 1966:5). Adaptation is an automatic consequence of natural selection and the acceptance of a non-teleological origin of life implies the acceptance of the key position of adaptation (Williams 1966).

Often two categories of adaptation are recognized: 'those that relate to the continued existence of the individual soma, and those involved in reproduction. Basically, however, all adaptation must relate to reproduction' (Williams 1966:159). Reproduction is of the essence (Haukioja 1982, Dawkins 1976).

The evolution of sex is one of the transcending events in the evolution of life (Maynard-Smith 1978, Ghiselin 1974, Williams 1975). Sexual reproduction inevitably entails adaptations (s.str. e.g., Williams 1966:9) to bring the gametes of biparental organisms together as a first step towards achieving syngamy. Syngamy is an extremely improbable phenomenon. Gametes are tiny specks in the vast biosphere - an immense environmental space - and yet they fuse with astonishing facility and regularity. The probability that gametes encounter one another by chance is remote in the extreme. Something must bring them together or improve the probability of encounter. It is widely accepted that mechanisms exist that serve this purpose or function. Adaptations exist for achieving syngamy.

Sexual reproduction has a very important corollary: it

automatically forms groups of individuals. But groups quite unlike classes in taxonomy and 'the distinction is not merely a matter of degree' (Dobzhansky 1951:259). The groups which arise because of the interaction between individuals of opposite sex are genetic species; they are actualities as opposed to arbitrary groupings of, for example, similar things. Genetic species do not occur among asexual organisms (Dobzhansky 1951) or in 'agamic complexes' (Babcock and Stebbins 1938). In Dobzhansky's (1951) opinion it was Lotsy's (1931) idea of the 'syngameon', defined as 'an habitually interbreeding community of individuals', that directed the attention of biologists in the 1930's to interbreeding communities (but see Mayr [1982:118-119] for an alternative opinion on Lotsy). These entities became known as Mendelian populations and were and continue to be treated as 'fundamental realities of the living world' (Dobzhansky 1951:262).

The important property of a genetic species as opposed to a taxonomic one is that there is interbreeding and gene exchange. Sewell Wright's (1932) term: 'field for gene combination' is apt. This concept is somewhat analogous to Dobzhansky's (1951:263) 'most inclusive Mendelian population' but the former is more compatible with our current knowledge of a generally less-particulate mode of inheritance (Williams 1966:61). 'The species as a field for gene recombination' (Carson 1957) epitomizes what most regard as the unequivocal characteristic of genetical species (Paterson 1985, Mayr 1976, Dobzhansky 1970, White 1978, Bush 1975).

It is generally agreed that sexual coadaptation plays a major role in the cohesion of 'fields for gene recombination' and therefore of species. 'It is of no use for a species to have individuals of two sexes, supplied with reproductive apparatus mutually adapted for their propogation, if the individuals are not endowed with suitable genetically determined impulses ... instincts, prompting them to desire one another' (Darlington 1964:335-336). It is almost axiomatic that adaptations for achieving syngamy inevitably delineate populations. All biparental organisms possess special

adaptations to achieve fertilization. The formation of species is an automatic consequence of the evolution of sex. However general this knowledge is, it is only Paterson (see below) who has developed it into a logically coherent species concept. Before I discuss Paterson's concept it is necessary to consider the orthodox understanding of the diversity of life; only then will it be clear how radically different species concepts can be.

The diversity of species is usually treated as the product of a process of life which is continuous historically, at least to the point of origin, but discontinuous laterally. Cultural attitudes towards the lateral discontinuity of life - the gaps between species - have a very interesting and important effect in contemporary evolutionary theory. As I have suggested above there has for millennia been a cultural predisposition to think that absolute extinction or obliteration of individuals or 'types' (supposed by many to be 'God's creatures') must be something heathenish and 'bad'. Hybridization has long been thought to dilute the 'perfect' characteristics of 'types', therefore it would follow that hybridization must be 'bad'. In terms of cultural aversion to extinction the significance of the 'penalty' of hybrid sterility has apparently had a greater impact than the benefits accruing from the occasionally robust intermediate (e.g., the mule).

In the English and French languages (at least) the same aversion is reflected in the terms associated with hybridization (or heterogeneous mixing). Notice the contrast between the pejorative terms: mongrel, mish-mash, hodge-podge, and bastard*; and the approbative terms: thoroughbred, pure-breed, sheer, purity, unadulterated, pristine and breeding true. The vigilant destruction of hybrids has been practised by animal and plant breeders ever since prehistoric man attempted to prolong the life (even if through generative

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* French pejoratives for heterogeneous mixing include: bâtard, sang mélangé, hybride and chimère.

cycles) of 'good' breeds; African cereal crops are a particularly convincing example (Pernes 1983). It is also worth noting how these linguistic metaphors and agricultural practices are in keeping with religious superstition e.g.: 'Thou shalt not let thy cattle gender with a diverse kind; thou shalt not sow thy field with mingled seed; neither shall a garment mingled of linen and woollen come upon thee.' (Leviticus 19:19). Hybrids have come to be thought of as 'bad' things in nature, no doubt this accounts for the popular opinion that hybrids ought to be quite rare and that 'natural selection should prevent hybridization'.

The 'perfection' of types or 'kinds' (species) in nature has been taken to be proof of the existence of a creator, a Grand Watchmaker (Paley 1828). Paterson (1982b) has shown how this could lead to the view that hybridization effectively dismantles God's handiwork. 'God forbids hybrids to breed' and 'the sterility of true hybrids afford another evidence of the jealousy with which the creator regards all attempts to introduce confusion into his perfect plan' are two quotes that reflected popular opinion in Darwin's time (Ellegard 1958:207) but which continue to taint theory (Paterson 1982b). The American naturalist and geologist, J.D. Dana, wrote in 1857 (Ellegard 1958:201): 'Species are units fixed in the plan of creation ... divine appointments which cannot be obliterated.' Here once again is an example of theism linked to eternal existence and the avoidance of extinction. These feelings have an ancient pedigree and are culturally inculcated. Paterson (1978, 1981, 1982b, 1985) has argued that these old theological concepts 'deceptively clad in new clothes' are at the foundation of current ideas about species and speciation.

The current paradigm with respect to species, how they evolve and what they are, is largely the product of two influential biologists: Dobzhansky and Mayr, who seem to have embraced a Wallacean (cf. Darwinian) version of change and progressive improvement through natural selection (Gould 1977; Paterson 1982a; Ghiselin 1969, 1974). A flurry of books in the late 30s and early 40s ushered in the so-called neo-Darwinian synthesis after a 'period from 1880 to 1940 when Darwin was

out of fashion' (Ghiselin 1974:30). 'What is remarkable about these works (Dobzhansky 1937, Mayr 1942), [and others of the same genre: Huxley 1942, Simpson 1944] is not their difference of opinion but rather their essential agreement on basic principles' (Carson 1957:23). Stebbins (1950) also falls within this group. This is how Dobzhansky (1937, 1951:180) saw species: 'Unlimited interbreeding of distinct species would result in submergence of the existing genetic systems in a mass of recombinations' and 'unrestricted hybridization would produce too many ill-adapted gene combinations. The arrays of genotypes which inhabit the adaptive peaks must be protected from disintegration'* (Dobzhansky 1951:261). Similarly Mayr too places great emphasis on the barriers to interbreeding: 'Isolating mechanisms are among the most important biological properties of species' writes Mayr (1963:109, Mayr and Provine 1980). Paterson (1982b) equates these types of statements to those made 80 years earlier by naturalists concerned with the 'confusion of all organic forms'.

From this type of thinking arises a concept and definition of species. Dobzhansky (1951:262-263) writes: 'Species are, accordingly, groups of populations the gene exchange between which is limited or prevented in nature by one, or by a combination of several, reproductive isolating mechanisms.' Similarly Mayr (1963:20) writes that 'Species are more unequivocally defined by their relation to non-conspecific populations ("isolation") than by relation of conspecific individuals to each other. The decisive criterion is not the fertility of individuals but the reproductive isolation of populations' and that 'this terminology emphasizes that the underlying concept is based on the biological meaning of the species, that is, to serve as a **protective device** for a well-integrated, co-adapted set of gene complexes' (my emphasis). Thus Mayr, in 1970, considered species to be 'Groups of interbreeding natural populations that are reproductively isolated

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* This argument is false because with unlimited interbreeding no diversity would ever have arisen to be broken down!

from other such groups' (but see amended wording: Mayr 1976:484). Many authors favour this concept of species which is known generally as the Biological Species Concept (Dobzhansky 1940; Mayr 1942, 1957, 1963:20, 1976:479) but which is called by Paterson (loc.cit.) the Isolation Concept - it conforms with deep-seated human predilections.

The field for gene recombination is, according to the Isolation Concept, delimited by reproductive isolating mechanisms. Such mechanisms are said to be adaptive, they are ad hoc mechanisms and as Mayr (1963) noted 'it is therefore somewhat difficult to comprehend how isolating mechanisms can evolve in isolated populations'. This is the crux of a serious flaw in the concept, a problem Mayr has never actually resolved (Mayr 1985) and indeed a problem for which there may be no solution (Muller 1939, 1942; Moore 1957; Paterson 1986). The usual speciation model (e.g., Ayala et al. 1974) proposed under the Isolation Concept requires that incipient species which have diverged genetically in allopatry, hybridize in secondary sympatry. It is then hypothesized that adaptive change to increase positive assortative mating leads to further genetic divergence between populations until they no longer hybridize. This is called 'speciation by reinforcement'. 'There is a distinct logical nexus between the term "Isolating Mechanism" and the Reinforcement Model of speciation' (Lambert and Paterson 1984:502). The species which result from this theoretical mode of speciation must be treated as adaptive devices; species are adaptive if they are shown to change in order to survive. Conceptually such species are fundamentally different to the types of species which result automatically after the evolution of sex (Paterson loc.cit., Carson 1987). Speciation by reinforcement rests on group selectionist assumptions, but these have been seriously undermined (Williams 1966).

The so-called 'reproductive isolating mechanisms' (Dobzhansky 1951:262, Mayr 1942) are usually divided into two broad classes: premating and postmating mechanisms (Mayr 1963, Dobzhansky 1970, White 1978, Grant 1971). Among the so-called postmating isolating mechanisms Mayr (1963:92) includes:

gametic mortality and hybrid inviability or sterility; but these cannot be directly selected. If sterility occurs in a hybrid it cannot be said to be 'a special endowment' (Darwin 1859). These are surely not mechanisms which evolved for the function (sensu Williams 1966:9) of reproductive isolation. They cannot be called ad hoc mechanisms for, as Darwin (1859:264) noted, they are 'incidental on other differences'. With respect to the other class (premating mechanisms), these are quite simply paradoxical. On the one hand it is generally accepted that nearly all speciation occurs in complete allopatry (Dobzhansky 1970, Bush 1975, Mayr 1963, White 1978). While on the other hand premating isolating mechanisms, in theory, can only evolve in sympatry. Otherwise they are not adaptive they are incidental effects (Gould and Vrba 1982). If they exist already in allopatry i.e. before secondary sympatry, they should be called isolating effects, not mechanisms nor adaptations (Paterson 1985).

What happens when two intersterile populations meet and hybridize? Paterson (loc.cit.) was first to notice that the hypothesis of speciation by reinforcement as it is usually formulated (Ayala et al. 1974, Avise 1976 and see above), is comparable to the well-known condition of negative heterosis. Paterson (loc.cit.) showed how, from the usual assumptions made in population genetics theory (Li 1955), it is possible to predict with simple deterministic algebra, that instead of reinforcement 'natural selection would act to eliminate the **cause** of the heterozygote disadvantage. This is much more likely' writes Paterson (1982a:55) 'than the reinforcement alternative, because it does not require the ready availability of appropriate reinforcement alleles ... nor does it encounter difficulties due to the coadaptation of the male and female components of the mate recognition system'. Additional difficulties with the reinforcement model are discussed in detail by Spencer et al. (1986) following Paterson (loc.cit.). Natural selection will either act to remove the cause of hybrid disadvantage or, when the selection coefficient (s) = 1, will act to eliminate the rarer population.

When hybrids have a selective coefficient between zero

and unity Paterson considered that there would probably be insufficient time for reinforcement to be completed before the source of the negative heterosis is eliminated (Lambert et al. 1984, Spencer et al. 1986, see also Wilson 1965). It is highly improbable that natural selection will select reproductive mechanisms to isolate two potentially miscible gene pools (Paterson loc.cit., Harper and Lambert 1983b, Lambert et al. 1984, Spencer et al. 1986). In other words it is not to be expected that the limits of a 'field for gene recombination' will be set by isolating mechanisms. It is predicted that when negative heterosis occurs natural selection will eliminate the cause of the hybrid disadvantage.

These are the considerations which motivated the studies reported on in this thesis: what happens in artificial hybrid swarms when maladapted mating behaviour is the cause of negative heterosis? The prediction is that the factors responsible for a lack of coadaptation between the sexes will be eliminated.

Because of the bias induced by the prevailing Isolation Concept, few studies have been made of hybrid populations which have not started with the preconception that natural selection will act to evolve reproductive isolation. A large number of attempts have been made to verify experimentally the model of speciation by reinforcement, see, for example, the studies of Koopman (1950), Kessler (1966), Crossley (1974), Wallace (1950, 1954), Knight et al. (1956), Kitagawa (1978) and Petit (1978, 1980), none of which have produced convincing evidence that, under natural conditions, reinforcement will occur (Paterson 1978). Often increased assortative mating was obtained but it was never shown to have become fixed or that it would persist without artificial interference. Indeed there was a tendency for it to revert to the original form when selection was relaxed. Paterson's prediction from population genetics theory (extinction of the smaller population when hybrids are sterile) was invariably prevented by the artificial maintenance of parity (of parental stock) each generation. Without interference the smaller population moved rapidly to extinction (Paterson 1978, Lambert and Paterson

1984, Spencer et al. 1986).

Restoration of mating behaviour to an original condition indicates that it possesses an intrinsic stability. The Specific-mate Recognition System (SMRS) is a subset of an overall system of mechanisms for achieving syngamy, the Fertilization System (Paterson 1980, 1985). By emphasizing the coadapted nature of the SMRS one is lead automatically to predicting that it will be stable. Whereas nothing in the Isolation Concept directs attention to this point (Carson 1987). The signal-response-signal-etc. chain of reaction between males and females (or their cells) is dynamically stable by virtue of its form, it works best when all signalling mechanisms impinge on all sensing and processing mechanisms. The absence of a response or a signal may, or may not, halt the chain of events leading to syngamy (Lambert 1984a). But it is certain that individuals in single populations are fitter when they possess sexually coadapted arrays of signals and responses. In such populations individuals which do not have a signalling system which is fully recognised by members of the opposite sex may be described as 'disadvantaged'. Thus artificial hybrid populations in which sexual behaviour is originally diverse are an appropriate model to test the prediction that natural selection will eliminate the cause of heterozygote disadvantage.

Drosophila are useful organisms to use in such ethological and evolutionary studies because their confinement in the laboratory apparently does not interrupt or unduly interfere with the operation of the SMRS. Their courtship progresses in the same way as is observed in nature (Spieth 1966, 1974; Ewing 1983). D. nasuta and D. kepulauana have been used in the present studies. They are morphologically very similar, both have a silvery white frontal pollinosity and a dark pleural stripe in males, while other taxa in the nasuta subgroup have only bands of pollinosity (e.g., D. sulfurigaster or D. pulaua) or no pollinosity (D. pallidifrons) and may lack pleural banding (D. niveifrons). A detailed principal component analysis of external anatomy

revealed no significant difference between nasuta and kepulauana (Kitagawa et al. 1982) and their genitalia, though distinctive in many other species, are indistinguishable (Wilson et al. 1969). They are thought to be very closely related phylogenetically (Lambert 1975, 1976, 1977; Wilson et al. 1969). They are cryptic species.

Karyotypically they are also similar. Populations of D. nasuta are usually 2n = 8 in India, Africa and on Indian Ocean islands but 2n = 6 in Thailand and Taiwan. Chromosome 3 and the X fuse to form a single, large metacentric in east Asian populations (Wakahama et al. 1983); the Y is J-shaped. Sajjan and Krishnamurthy (1972, Appendix A) divided the species into two subspecies: D. n. nasuta (2n = 8) and D. n. albomicana (2n = 6); the two are completely interfertile despite the karyotypic differences (see below). Drosophila kepulauana has 8 chromosomes; the Y is rod-shaped and the dots have slightly more heterochromatin (Wakahama et al. 1983).

Polytene studies have revealed a very high degree of inversion polymorphism in nasuta. Lin et al. (1977) report 91 heterozygous inversions in Taiwan isolines and Ranganath and Krishnamurthy (1975b) report 35 inversions in Indian isolines. D. kepulauana is relatively uncommon and consequently it has not been studied to the same extent (Clyde 1982).

Drosophila nasuta is widespread in the Afrotropical and Oriental biogeographic regions; in this study it is reported for the first time from southern Africa where it was found in Swaziland. Drosophila kepulauana is restricted to the Borneo region (Lambert 1975, 1976; Wilson et al. 1969). There is no evidence of natural sympatry near Singapore where the two ranges nearly overlap; only kepulauana has been reported near the southern tip of the Malay Peninsula while 290 km north, only nasuta has been found (Fig. 3, p. 32); none have been reported from in between.

The sexual behaviour of males of the two species differs distinctively. Firstly it has been found that the daily sexual activity pattern of nasuta peaks suddenly at simulated dawn and that this coincides to a peak of matings in the first c. 15 min of a 12 h light phase. This activity pattern is male-

dependent because the pattern remains more-or-less unchanged when the type of female is varied. In contrast, kepulauana males do not mate at all in the first 15 min; there is a relatively depressed peak in the temporal copulation pattern at c. 60 min which attenuates only gradually thereafter.

Neither species was observed mating in the dark despite the fact that in the presence of virgin females nasuta males, for example, responded by initiating courtship; they were unable to maintain orientation and failed to mate. Light is apparently needed in the penultimate stages of the signalling 'volley' between males and females. Lambert (1982) has suggested that the frontal pollinosity and lateral stripe are important components of the SMRS of these species and that the male's courtship movements enhance visibility. Nevertheless, Wright (1977a,b; Ewing 1983) has evidence that under crowded conditions and complete darkness some fertilization eventually occurs after very prolonged periods. It is probable that in their normal or preferred habitat light plays an important part in the SMRS of both species.

Indirect evidence suggests that these flies also transmit and receive signals via tarsi and that such signals are not species specific; nasuta and kepulauana males orient to nasuta and kepulauana females indiscriminately after touching their tarsi*. There is evidence, however, that tarsal substances are sex-specific i.e. part of the SMRS. Virgin females elicit greater sexual activity among males than do recently-mated females; this excitation commences when the tarsi of virgin females and males come into contact. Freshly mated females are unattractive. These observations suggest that the odour of females changes throughout their adult life and that males are adapted to sense these signals.

It has previously been reported that D. nasuta produces a distinctive sound with its wings during the circling phase of

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* It is more likely that a chemical rather than a tactile signal is involved given that cuticular hydrocarbons have been found on D. melanogaster legs (Jallon et al. 1986).

courtship (Leroy and Eucher 1981). In the present work it has been discovered that kepulauana males are silent. During wing-extension their wings move through a greater arch and much more rapidly and I have suggested that the physical dynamics of the wing's 'out-thrust' prevent vibration. The wing probably strikes the sensors of the head or forelegs of the female, whereas, as far as can be detected, nasuta males do not touch females with their wings. No intraspecific differences have been found in the acoustic signals produced by nasuta males from Thailand or Taiwan populations on the one hand and Swaziland, Indian or Malagasy populations on the other (results not presented). Thus, despite the great expanse of the range (sub-cosmopolitan) and the karyotypic and cytological differences the SMRS of nasuta is stable over the breadth of its range (see Henderson and Lambert 1982).

The courtship pattern (the bodily and wing movements) of males of the nasuta subgroup of species has been the subject of several previous studies (Spieth 1969; Lambert 1982; Wright 1977a,b; Leroy and Eucher 1981). The most important visual differences between the nasuta and kepulauana courtship are the positions in which the males commence the wing display (the 'starting position'), the duration of time which punctuates consecutive wing displays (the interburst-interval) and the sound produced by the males during wing displays. D. kepulauana has a more rapid interval between discrete components of the display although bout-lengths in both species are similar (c. 9.5-10.0 s). Males alter their speed when females move and this suggests that periodicity is more important than quantity during courtship displays.

There is evidence that the SMRS of nasuta and kepulauana comprise a variety of visual, tactile, chemical, temporal and acoustic signalling components. Certain signals elicit similar responses in both species e.g., the signals transferred via the tarsi of virgin females. While other signals elicit different responses e.g., acoustic and frequency related phenomena and the tactile or chemical signals on males' legs.

Under confined and artificial conditions it is possible to hybridize nasuta and kepulauana. More F_1 progeny reach

maturity when they are derived from nasuta females. The F_1 nasuta/kepulauana hybrids are fertile and the kepulauana/nasuta hybrids are normally sterile. Mating occurs more freely between nasuta females and kepulauana males than vice versa; this asymmetry complicates the interpretation of hybrid behaviour and is one of the reasons for concluding that nasuta females are responsive to a broader range of signals.

Although many aspects of F_1 courtship are intermediate, a striking exception is the temporal mating pattern of F_1 hybrids which peaks in the first 15 min very like nasuta and most unlike kepulauana; it cannot be described as intermediate. This is a very puzzling result. It is important because in certain advanced hybrid populations (13 and 39) it has been shown that the '15-min peak' diminishes considerably (Fig. 38) and that this is associated with an overall restoration of the sexual behaviour pattern to a signalling system efficient only with kepulauana females. In other populations, the males of which are sexually efficient only with nasuta females, the '15-min peak' has been retained.

As is often the case in first generation hybrids there is very limited variability between individuals, they usually have intermediate characteristics. Fitness may increase or decrease depending on the habitat (Anderson 1949). Hybrids are often more vigorous. In the present study the nasuta/kepulauana hybrids are large and active flies, a heterotic effect noticed also by Spieth (1969). Vetukiev (1953, 1954) discovered similar increased vigour and limited variability in F_1 hybrids of various other Drosophila species.

It has been shown in the present study that nasuta/kepulauana hybrid males devote more of their available time to courting than do either of the parental species. It has also been shown that this is to no avail i.e. despite the greater quantity of courtship they fail to achieve the numbers of copulations normally achieved by either of the parental males. In addition it has been found that F_1 hybrid females respond positively more often to males which have signalling characteristics like nasuta or kepulauana than to their male siblings. It may be concluded that male and female F_1 hybrids

are not sexually coadapted. In nature F_1 hybrid females would probably mate quite readily with either kepulauana or nasuta males, if they were courted by them (and this is not certain); in contrast F_1 hybrid males would probably not mate at all.

It has been clearly demonstrated that the courtship of F_1 hybrids is very inefficient with kepulauana females. In contrast, F_1's score surprisingly high numbers of copulations with nasuta females when the females have 'no-choice'. However, when nasuta females are among both nasuta and F_1 males i.e. in a so-called 'choice' situation, few hybrid males succeed in mating. The conclusion is that nasuta females respond to a broader range of signals than do kepulauana females but that they respond more often to conspecific signalling than to any other form. Non-hybrid females move away from F_1 males disproportionately more often than they do from conspecifics, these 'rebuttals' are even more marked after the hybrid male has issued a wing-extension. The wing-extension in F_1 hybrids is always associated with an abnormal acoustic signal, about half the duration of a nasuta burst.

The fertile F_1 nasuta/kepulauana hybrids were the founding individuals in the hybrid populations used in this study. F_2 hybrids, in marked contrast to their F_1 hybrid parents, were very variable and less vigorous. Once again these results resemble those of Vetukiev (1953, 1954) in interspecific hybrids of the D. willistoni group. This is the outcome among numerous other animal and plant hybrids, many examples of which are discussed by Anderson (1949) and Darlington (1958).

In the present study second filial hybrids were shown to have various combinations of behavioural characteristics typical of nasuta or of kepulauana. For example, some F_2 hybrid males started courtship from a position diagonally at the rear like nasuta while others started directly in front like kepulauana; the majority started from an intermediate position typical of F_1. This phenotypic pattern resembles a 1:2:1 ratio suggesting that its determination involves two alleles and not polygenes.

Timing parameters were also variable and were independent

of other components in the overall sexual signalling system. In certain instances F_2 males lunged like kepulauana but the ibi was prolonged and closer to the ibi of nasuta. Other second generation hybrid males circled like nasuta but were silent like kepulauana. Various other novel combinations were observed but the predominant courtship form in the second generation was like the intermediate F_1

The conclusion is that the different components of courtship are controlled by independently segregating genes. The intermediacy of first generation hybrids and the variety in the second generation implies that overall courtship is not under the control of a single genetic factor. There is a very broad range of fitness in F_2 hybrids upon which natural selection acts and there is an absence of dominance except perhaps with respect to the temporal mating pattern.

Finally, advanced hybrids (c. F_{20-35}) were placed with non-hybrid females in a series of mating experiments. The objective was to determine what type of signalling system was operating within each hybrid population after a long period of natural selection. Were the males still sexually inefficient and maladapted to siblings like F_1 and F_2 males? Had the heterogeneous behavioural elements that were visible among early hybrids reassembled under natural selection?

If sexual signalling systems in advanced hybrid populations revert to the nasuta SMRS, for example, then it is expected that advanced hybrid males, with such behaviour, will achieve as many matings in a standard time as do nasuta males among nasuta females. Indeed if the sexual behaviour is the same as that of nasuta then it should have all the characteristics: there should be a peak at 15 min in the temporal mating pattern and a greatly reduced efficiency with kepulauana females (i.e. an Efficiency Index of 0.00). Similarly if the maladapted F_1 and F_2 sexual signalling system is reconstituted in the direction of kepulauana then the advanced hybrids would be relatively efficient with kepulauana females; they would have a temporal mating pattern like kepulauana (cf. nasuta) and they would have an Efficiency Index of 1.00.

The outcome in the 48 hybrid populations is that 67% acquire a nasuta SMRS, 4% acquire a kepulauana SMRS and 29% have reached an equilibrium condition wherein males are inefficient with both kepulauana and nasuta. Given that neither nasuta nor kepulauana females were present at any time during the many generations of culturing, the restoration of signalling systems to non-hybrid states occurs as a result of natural, intrinsic, population dynamic and homeostatic phenomena alone.

Selection against the less fit is like neutralization. There are a number of ways in which natural selection neutralizes the effects of hybridization. Hybrids are generally unfit but their fitness is variable and this variability has a genetic basis (Anderson 1949) thus one should expect the fittest hybrids to prevail in hybrid swarms. It should be emphasized that, in this sense, predicting the neutralization of infertility is not at all the same as proposing that natural selection provides a means for limiting hybridization. Neutralization and limitation (or prevention) are two quite different phenomena. Williams (1966:30-32) makes clear a most relevant point in this respect in his attack of group selectionist thinking: 'The possibility that populations can take special steps in response to the threat of imminent extinction is often implied in elementary biology texts... [but natural] selection has nothing to do with what is necessary or unnecessary or what is adequate or inadequate for continued survival. It deals only with an immediate better-vs-worse within a system of alternative, and therefore competing, entities. It will act to maximize the mean reproductive performance' i.e. the mean fitness will rise from natural selection acting on individuals.

Thus if hybrids occur in nature one should only expect that they be subjected to the same selective pressures as other competing individuals in the same system and that even if hybridization leads to the demise of a population, individuals in that population should not be expected to respond by avoiding matings which lead to a below average reproductive performance. 'The interspecific explanations

should emerge as phenomena of last resort, since the commonest problem that faces each species is reliable communication among its own members in a noisy channel' (Hailman 1977:306).

What evidence is there that neutralization rather than prevention occurs in nature. Perhaps the most important indirect evidence is that even where there has been extensive hybridization for hundreds or thousands of generations we continue to find living hybrids - hybrid swarms (Mayr 1963). This is clearly not evidence of the evolution of 'preventive measures'. Evidently the deleterious effects of negative heterosis have been neutralized in established hybrid zones. I view neutralization as something similar to becoming adapted to the hybrid condition and prevention as the acquisition of *ad hoc* contrivances for avoiding it.

The evidence from modelling reveals some very real difficulties with the idea that 'prevention of hybridization' can evolve under natural conditions. The thorough analysis of Spencer *et al.* (1986) led them to conclude that a series of stringent assumptions must be met for reinforcement (i.e. prevention) to work: reproductive characters must be variable, selection must be intense to counteract the inherent instability and chance of extinction, hybrid fitness must be zero or close to it and because the selective pressure for assortative reproductive characters becomes weaker as these characters are established, selection alone cannot complete the process. 'These imply that speciation by reinforcement is not nearly as prevalent as much of the biological literature suggests' (Spencer *et al.* 1986). Lambert *et al.* (1984) determined a natural rate-of-change constant, *k*, that must be 0.15 or greater for reinforcement to evolve between divergent systems of specific-mate recognition. They then undermine an experiment (Crossley 1974) that was designed to illustrate the divergence between *D. melanogaster* strains by showing that the *k* value was only 0.016. Moore's (1957) arguments against 'prevention' (i.e. the evolution of isolating mechanisms) are particularly compelling. Apart from a number of case studies he raises the serious theoretical criticism that the selection of isolating mechanisms in a hypothetical population A at a

hybrid zone with B would have the effect of alienating A individuals from other A's away from the zone.

Plants neutralize or regularize meiotic problems in hybrids with polyploidy, vegetative growth, apomixis or any combination of these processes (Darlington 1958). Babcock's (1947) studies of Crepis demonstrated that the several diploid 'species' representing 'extremes of morphological and ecological variation ... are connected to each other by means of a vast superstructure of polyploids' (Stebbins 1966:1466). The hybrid origin of polyploidy is considered to be a general phenomenon in plants (Stebbins 1966, Grant 1971). 'The polyploid state serves to restore fertility ... in this way a fertile and stable intermediate form is evolved' (Stebbins 1966:1467). On the other hand sexuality may be dispensed with altogether. Dandelions (Taraxacum) are usually triploid apomicts. Triploidy is derived from a cross between 4n and 2n individuals. When triploidy arises - and there is no reason to suppose that this is a rare occurrence - in one stroke it removes the possibility of meiotic reduction and the need for fertilization (Darlington 1958:160). 'This capacity (of the egg to develop without fertilization) gives the sterile polyploid or the sterile mutant **a means of escape from its sterility**' (Darlington 1958:161, my emphasis). Apomixis becomes obligatory in triploid Dandelions but it also neutralizes the negative effects of hybridity. Problems associated with chromosomal irregularities in diploid hybrid organisms are neutral in apomictic plants and parthenogenetic animals. 'Apomixis is an escape ... guided in one or in several steps by natural selection; but it is an escape' which according to Darlington (1958:168) 'leads only to extinction.' But parthenogenesis need not be an evolutionary dead-end (Suomalainen and Saura 1973). 'In comparison with sexual reproduction, a transition to parthenogenesis gives a formidable advantage in reproductive efficiency at the cost of a slowed-down rate of evolution'.

Further evidence that the switch to parthenogenesis or asexuality is a solution to the problem of hybridization comes from zoological studies. Parthenogenesis is known in nearly

all animal groups (Suomalainen 1962) and many appear to have a hybrid origin (Hewitt 1975). Parthenogenesis is often associated with polyploidy and it results in great stability. The Australian Eumastacid grasshopper Warramaba virgo (see below), the frog Rana esculenta (Maynard Smith 1986), psocid insects (Mockford 1971), the lizard genera Cnemidophorus and Lacerta (Darevsky and Danielyan 1968, Lowe et al. 1970), the black fly Cnephia mutata (Basrur and Rothfels 1959), curculionid beetles (White 1970, Smith 1971), the fish genera Poecilia and Poeciliopsis (Abramoff et al. 1968, Schultz 1969) and the salamander jeffersonianum complex (Uzzell and Goldblatt 1967), are all parthenogenetic and of probable polyphyletic origin.

Parthenogenesis is an escape from the deleterious effects of hybridity. For example, the 'Parthenogenetic Otiorrhynchus (Curculionidae: Insecta) populations are free of any possible complications due to interbreeding ... By dispensing with recombination these [parthenogenetic polyploid] populations have also **rid themselves of the genetic load** of inferior allele combinations' (Suomalainen and Saura 1973:507, my emphasis).

If natural selection removes the cause of hybrid disadvantage as Paterson (1978, 1982a, 1985) predicts then it should be possible to find natural systems which are viable and have a hybrid origin, systems in which the effects of negative heterosis are neutralized. Moran and Shaw (1977) found cytological evidence that the grasshopper, Caledia captiva, eliminates disadvantageous elements in wild populations following hybridization. With respect to the Vandiemenella viatica complex they have shown, empirically, that a parapatric zone of disadvantaged hybrids acts as a 'semipermeable filter' holding up deleterious rearrangements at the interface while allowing shared chromosomes to pass through. Thus the boundary between the two populations is delimited by the rearrangement only (Paterson 1978, 1981).

Warramaba virgo is a diploid all-female species which reproduces parthenogenetically; it has been the object of a number of cytological and evolutionary studies in Australia

(White and Contreras 1979). Originally hybridization was dismissed as the mechanism for its genesis because of chromosomal homozygosity (White and Webb 1968, White 1970) and because no putative ancestral populations were known. However, the discovery in Western Australia of the race P196 allowed Hewitt (1975) to confidently conclude that W. virgo had a polyphyletic origin. It has also been noted that there is frequent hybridization between different cytological races of this species and that the stable systems resulting vary (Webb et al. 1978). There is no evidence that reproductive isolating mechanisms have evolved. Once again these observations are best interpreted as indicating 'neutralization of the negative effects of hybridity'.

Stalker (1954) identified 23 species of Drosophilidae which could reproduce, at a low rate, parthenogenetically, only three of these produced adult progeny. Additional species have been added to the list since 1954 (summarized in Suomalainen 1962:361). But there are no examples from nature of polyploidy in Drosophilidae. However there is evidence that neutralization results not from parthenogenesis but by elimination of elements which cause negative heterosis.

Natural selection may neutralize the effects of hybridization by eliminating chromosomes which are deleterious in hybrids. The cytology of hybrids between the two nasuta subspecies D. nasuta nasuta (2n = 8) and D. nasuta albomicana (2n = 6) (Sajjan and Krishnamurthy 1972, Appendix A) has been extensively studied (Ranganath and Hägele 1982; Ranganath et al. 1982; Hägele and Ranganath 1982, 1983; Ramachandra and Ranganath, 1986). Ramachandra and Ranganath (1986) established artificial hybrid swarms using the fertile progeny from the cross: n. albomicana/n. nasuta or the reciprocal. The action of natural selection over some 20 generations led to the evolution of two new and stable karyotypic strains which they named Cytoraces I and II. In Cytorace I the hybrid karyotype stabilized with males having 2n = 7 and females 2n = 6. While in Cytorace II both males and females had 2n = 6. The reconstituted karyotypes comprised chromosomes from both parental strains. They write: 'Even though the chromosomes of

the parental races are duly represented in the F_1, there is selective retention/elimination of certain chromosomes in the succeeding generations during which repatterning of the karyotype has taken place' (Ramachandra and Ranganath 1986:243). Both hybrid strains are characterized by the gradual elimination from generation to generation of the maternally inherited dot chromosomes and the n. nasuta X chromosome. Thus identifying these elements as the causes of negative heterosis among F_2 and subsequent generations (Ramachandra and Ranganath 1986). 'Thus, hybridization has resulted in establishing a new mixed karyotype' which is stable and viable and which, importantly, does not restrict population introgression (Ramachandra and Ranganath 1986).

In a similar study of the closely related species D. arizonensis and D. mojavensis, Mettler (1957) was able to show that the arizonensis X chromosome was consistently eliminated; this identifies it as the disadvantageous element in the hybrid gene pool. In the region where the species pair exist in sympatry, reproductive isolation is highest (Wasserman and Koepfer 1977). This is cited as evidence of reproductive character displacement (Markow 1981, Markow et al. 1983). However, Zouros (1986) and Vigneault and Zouros (1986) have shown that particular chromosomal combinations effectively suppress sterility while others cause it. Therefore it is possible that the behavioural variability observed among allopatric and sympatric strains is an incidental consequence of natural selection differentially eliminating chromosomes from the hybrid gene pool in the zone of overlap. Natural selection acts by eliminating the cause of hybrid disadvantage; it 'neutralizes' the negative heterotic effects rather than generating 'preventive' (premating) mechanisms.

The genetic rescue of inviable Drosophila hybrids has recently been the object of detailed genetic analysis (Watanabe 1979, Coyne 1984, Coyne and Kreitman 1986, Hutter and Ashburner 1987). The rare hybrids of D. melanogaster and D. simulans are usually all female and sterile, males die during development. The occasional male survives and is XO, the product of primary non-disjunction. However, another cause

of male survival, called 'lethal hybrid rescue' (Lhr), is known; it has a genetic basis and was traced to chromosome 2R in the simulans genome (Watanabe 1979). Hutter and Ashburner (1987) found a similar gene in the melanogaster genome and called it 'hybrid male rescue' Hmr (Hutter and Ashburner 1987). Remarkably, Hmr also rescues the otherwise inviable hybrids between melanogaster females and mauritiana and sechellia males (Hutter and Ashburner 1987); the latter two species are closely related. Thus, a gene exists which has the effect of restoring viability in hybrids and it is functional in different contexts. Once again we have evidence here of natural selection working to neutralize the effects of negative heterosis. It is unlikely that Hmr^{+} has evolved to ensure the inviability of the species hybrids because it is difficult to see how such an allele could become fixed in a species (Hutter and Ashburner 1987; Spencer et al. 1986; Moore 1957; Paterson 1978, 1985).

Finally there is evidence that behavioural elements which reduce hybrid fitness are eliminated from hybrid populations. Wallace et al. (1983) found that in hybrid populations 'the relatively deleterious recessive alleles are selectively eliminated' (Wallace et al. 1983:472). They refer to the genetic basis of sexual behaviour and conclude that 'members of (such hybrid) populations need not adopt one or the other of the ... behaviors of the parental species; instead, by using the various elements ... they can construct systems that are demonstrably different from each other and from the originals' (Wallace et al. 1983:476).

If the same elements are always eliminated one would not expect variability in the outcome, unless chance plays a part. It was found in the present study and by Wallace et al. (1983), that mating systems stabilize in different ways. Therefore chance is important; it affects context and opportunity. A state of behavioural flux exists when elements segregate in early generations. Random drift can take a certain element to extinction thus providing the opportunity for a fertilization system, lacking that element, to evolve. Results further suggest that elements of both parental strains

may be lost. The resulting fertilization system, though functional within the population, is relatively inefficient among nasuta or kepulauana flies. 'Historical opportunity ... controls the direction' (Jacob 1977:1166), where there is no opportunity there can be no recovery. The loss of vital elements prevents the evolution of particular behavioural repertoires. Natural selection 'tinkers' with what is available (Jacob 1977).

In the present work most early-filial hybrid females are relatively indiscriminate having a broad, nasuta-like responsiveness to a variety of male signals. The remaining females have narrow, kepulauana-like sensitivity, as a consequence they have low fitness. Bearing in mind that hybrid males generally court vigorously and indiscriminately the variability in females is a possible explanation for the recovery, in 67% of the populations, of the nasuta SMRS (cf. 4% kepulauana). Furthermore, this suggests that when asymmetrical mating exists, the behavioural outcome after introgression will be biased towards the parental type having the broader sensitivity. This is supported by the observation (data not presented) that after introgression between D. sulfuriqaster and D. pulaua, the sulfuriqaster SMRS was restored (see also Wallace et al. 1983 - 'Phyps-1' reconstructs the D. persimilis mating system).

The reorientation of mixed SMRS's to original positions implies that these represent stable states among the various possible configurations. It implies intrinsic linkage or cohesiveness. Anderson (1949:55) suggests that 'We may think of linkage ... either as a negative force that keeps new recombinations from appearing, or as a positive force tending to bring the hybrid population back to something very like the original types ... Linkage, in other words, takes what would have been a spherical mass of probabilities and draws them out towards the original positions' (Anderson, 1949:55). He continues (1949:56) 'positive pull back ... is stronger. It is, therefore, more effective to think of linkage as a factor of racial and specific cohesion rather than as a barrier between species and between races.' Sexual coadaptation,

together with adaptation to habitat, is the 'linke
genetic species. It is most unlikely that hybri
generates new mating systems with the purpose of p
further hybridization.

Hybridization can initially be destabilizing but not necessarily a dead-end. In this chapter are listed a number of cases which demonstrate how disadvantages are neutralized when natural selection eliminates causes of negative heterosis. My work on sexual behaviour conforms with these findings in plants and animals. I have also presented evidence that there is an increase of coadaptedness between the sexes through time and I conclude that this is due to the selective elimination of maladapted components. Once this perspective is attained it can be seen that natural selection overcomes the effects of hybridization in a number of ways. Various works on introgressive hybridization, the present behaviour work together with that of Wallace et al. (1983) show the same phenomenon. All support the 'elimination of cause' hypothesis as Paterson (1985) suggested. Taken together with the failure of the reinforcement model of speciation to make any headway, it now seems only reasonable to adopt the Recognition Concept. In future it will be difficult to justify the expectation of disruptive selection and reinforcement of divergence after hybridization, as has been done in the past.

Death is something profoundly disturbing for man because it is our certain fate; we do what we can to avoid it. Inevitably we project this preparedness and foresight into nature as we do with other realms of our consciousness. Such animist projection taints our knowledge of life (Monod 1970). For example, isolating mechanisms are proposed because it is thought that nature must somehow conserve what she has. But the fixity of nature is at variance with Darwin's (1858:460) view that from life's 'beginning endless forms ... have been, and are being, evolved'. Along that dark, former route one was led to think of hybridization as a muddying phenomenon - a process of ungodly undoing. Instead of looking for creativity after hybridization, biologists searched for prevention before it. This represented an erroneous projection. When organisms

introgress the interest should be in how they adapt in order to reproduce because reproduction is of the essence. Witness its spontaneity when humans forget themselves:

> 'On 11 November 1918 the Allied peoples burst into rejoicing ... As evening fell, the crowds grew more riotous. Total strangers copulated in public - a symbol that life had triumphed over death'
>
> (Taylor 1963:251).

APPENDIX A

Taxonomy of the species which have been placed in the Drosophila nasuta subgroup or which have been identified as synonyms. The D. nasuta subgroup belongs to the D. immigrans species group of the subgenus : Drosophila (Drosophilidae: Insecta). Detailed taxonomic bibliographies are not given here but may be found in Wheeler (1981, 1986) and Wilson et al. (1969, marked * below). Subgroup and species group categories follow Bock and Wheeler (1972).

D. albomicans Duda, 1923:43,47,48; 1924a:209; 1924b:245, Fig. 70; 1926:83, 88-89; 1940:23. = D. nasuta Lamb, 1914:346 (Sajjan and Krishnamurthy 1972).

D. albovittata Duda, 1926:87. = D. sulfurigaster (Duda, 1923).*

D. bilimbata Bezzi, 1928:159. = D. sulfurigaster (Duda, 1923).*

D. kepulauana Wheeler, 1969:219. (3122.3 is the type culture, see Table 3, p. 49). syn. D. nasuta kepulauana Rajasekarasetty, Ramesh & Krishnamurthy, 1977:125.

D. kohkoa Wheeler, 1969:217.

D. ?kohkoa McEvey & Bock, 1982:682. Possibly D. niveifrons Okada & Carson, 1982.

D. komaii Kikkawa & Peng, 1938:525. = D. nasuta Lamb, 1914.*

D. nasuta Lamb, 1914:346. syn. D. albomicans Duda, 1923 (Sajjan and Krishnamurthy 1972). syn. D. komaii Kikkawa & Peng, 1938.*

D. nasuta albomicana Sajjan & Krishnamurthy, 1972:60.

D. nasuta kepulauana Rajasekarasetty, Ramesh & Krishnamurthy, 1977:125. = D. kepulauana Wheeler, 1969 (Rajasekarasetty et al. 1977).

D. nasuta nasuta Lamb, 1914:346.

D. n eonasuta Sajjan & Krishnamurthy, 1972:60. syn. D. neonasuta Sajjan & Krishnamurthy, 1973:267 (Wheeler, 1981). = D. sulfurigaster (Duda, 1923) (Ranganath and Krishnamurthy 1977).

D. niveifrons Okada & Carson, 1982:407. See D. ?kohkoa (McEvey and Bock, 1982:682).

D. nixifrons Tan, Hsu & Sheng, 1949:202.

D. pallidifrons Wheeler, 1969:219.

D. pulaua Wheeler, 1969:218.

D. setifemur Malloch, 1924:351 redescription Clark, 1957:221. = D. sulfuriqaster (Duda, 1923).*

D. spinofemora Patterson & Wheeler, 1942:104. = D. sulfuriqaster (Duda, 1923).*

D. sulfuriqaster (Duda, 1923:48) (Spinulophila); 1926:83, 87-89. Absolute synonym of D. albovittata Duda, 1926:87, unjustified substitution for sulfuriqaster. syn. D. spinofemora Patterson & Wheeler, 1942.* syn. D. neonasuta Sajjan & Krishnamurthy, 1972 (Ranganath and Krishnamurthy 1977). syn. D. willowsi Curran, 1936.* syn. D. setifemur Malloch, 1924.*

D. sulfuriqaster albostriqata Wheeler, 1969:217.

D. sulfuriqaster bilimbata Bezzi, 1928:159 (as species). syn. D. spinofemora Patterson & Wheeler 1942:104.*

D. sulfuriqaster neonasuta Ranganath and Krishnamurthy, 1977:81.

D. sulfuriqaster sulfuriqaster (Duda, 1923:48). syn. D. setifemur Malloch, 1924:351.* syn. D. willowsi Curran, 1936:42.*

D. willowsi Curran, 1936:42. = D. sulfuriqaster (Duda, 1923).*

A series of undescribed taxa are referred to as follows: Taxon A a probable synonym of D. nasuta. Taxon B 'with white orbits in Cebu' (Mather and Thongmeearkom 1973:60) a probable synonym of D. sulfuriqaster. Taxon C 'with no white on the face in Taiwan' (Mather and Thongmeearkom 1973) a probable synonym of D. pallidifrons. Taxon D 'with a white face in Cebu' (Mather and Thongmeearkom 1973) a probable synonym of D. kohkoa. Taxon E 'with no white on the face... much smaller than Taxon C' (Mather and Thongmeearkom 1973) possibly a new species. Taxon F 'D. pallidifrons and Taxon-F (a new species captured in Kuching, Malaysia) have no whitish mark on the male frons' (Kitagawa 1978).

APPENDIX B

Table 1. Drosophila nasuta **control scores and Efficiency Indices**

	EFF. INDEX	TEST CODE	SCORE kep	SCORE nas	SIGNIFICANCE RATINGS 801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821 ***	822
nas	0.00	801	0	46		-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
nas	0.00	802	2	46	-1		-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
nas	0.00	803	2	47	-1	-1		-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
nas	0.00	804	2	45	-1	-1	-1		-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
nas	0.00	805	2	34	-1	-1	-1	-1		-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
nas	0.00	806	3	48	-1	-1	-1	-1	-1		-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
nas	0.00	807	7	48	-1	-1	-1	-1	-1	-1		-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
nas	0.00	808	1	45	-1	-1	-1	-1	-1	-1	-1		-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
nas	0.00	809	1	49	-1	-1	-1	-1	-1	-1	-1	-1		-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
nas	0.00	810	5	48	-1	-1	-1	-1	-1	-1	-1	-1	-1		-1	-1	-1	1	1	1	1	1	1	1	-1	1
nas	0.00	811	2	50	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1		-1	-1	1	1	1	1	1	1	1	1	1
nas	0.00	812	0	50	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1		-1	1	1	1	1	1	1	1	1	1
nas	0.00	813	3	46	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1		1	1	1	1	1	1	1	-1	1

Results lie within (-1) or outside (1) the 0.05 confidence interval of the combined control scores.
*** the 821 control scores are excluded in the calculation of Efficiency Indices (see text).

APPENDIX B

Table 2. Drosophila kepulauana **control scores and Efficiency Indices**

	EFF. INDEX	TEST CODE	SCORE kep	SCORE nas	SIGNIFICANCE RATINGS 801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821 ***	822
kep	1.00	814	25	5	1	1	1	1	1	1	1	1	1	1	1	1	1		-1	-1	-1	-1	-1	-1	-1	1
kep	1.00	815	24	6	1	1	1	1	1	1	1	1	1	1	1	1	1	-1		-1	-1	-1	-1	-1	-1	-1
kep	1.00	816	30	9	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1		-1	-1	-1	-1	-1	-1
kep	1.00	817	31	9	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1		-1	-1	-1	-1	-1
kep	1.00	818	23	17	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1		-1	-1	-1	-1
kep	1.00	819	20	18	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1		-1	-1	-1
kep	1.00	820	26	22	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1		-1	-1
kep	***	821	16	27	1	1	1	-1	-1	1	-1	1	1	-1	1	1	-1	-1	-1	-1	-1	-1	-1	-1		-1
kep	1.00	822	28	31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	-1	

Results lie within (-1) or outside (1) the 0.05 confidence interval of the combined control scores.
*** the 821 control scores are excluded in the calculation of Efficiency Indices (see text).

APPENDIX B

Table 3. Hybrid populations with Efficiency Indices between 0.00 and 1.00

	EFF. INDEX	POP CODE	SCORE kep	SCORE nas	SIGNIFICANCE RATINGS 801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821 ***	822
hyb	0.00	F1.a	2	42	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.05	F1.b	15	46	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	-1
hyb	0.05	02	2	47	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.05	17	6	42	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	-1
hyb	0.05	18	6	42	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	-1
hyb	0.10	16	4	36	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	-1	1	-1	-1
hyb	0.20	09	7	37	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	-1	-1	-1	-1	-1
hyb	0.20	33	8	38	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	-1	-1	-1	-1	-1
hyb	0.20	37	9	39	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	-1	-1	-1	-1	-1
hyb	0.20	49	15	40	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	-1	-1	-1	-1	-1
hyb	0.20	50	12	40	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	-1	-1	-1	-1	-1
hyb	0.25	20	8	30	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	-1	1	1	1	1	-1	-1	-1	-1	-1
hyb	0.55	48	18	30	1	-1	-1	-1	-1	-1	-1	-1	1	-1	1	1	-1	1	-1	-1	-1	-1	-1	-1	-1	-1
hyb	0.65	43	1	24	1	1	1	1	-1	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	1	-1	1
hyb	0.70	10	7	25	1	1	1	-1	-1	1	-1	1	1	1	1	1	-1	1	-1	1	1	-1	-1	-1	-1	-1
hyb	0.75	11	4	24	1	1	1	1	-1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	1
hyb	1.00	13	10	24	1	1	1	1	-1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1
hyb	1.00	39	18	23	1	1	1	1	-1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Results lie within (-1) or outside (1) the 0.05 confidence interval of the combined control scores.
*** the 821 control scores are excluded in the calculation of Efficiency Indices (see text).

APPENDIX B

Table 4. Hybrid populations with Efficiency Indices of 0.00

	EFF. INDEX	POP CODE	SCORE kep	SCORE nas	SIGNIFICANCE RATINGS 801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821 ***	822
hyb	0.00	01	1	50	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	03	3	45	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	04	7	48	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	05	2	41	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	06	7	42	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	-1
hyb	0.00	07	2	41	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	08	3	48	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	12	2	34	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	14	0	44	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	15	3	43	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	19	1	41	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	21	3	43	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	22	5	44	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	24	5	49	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	25	1	48	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	26	4	46	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	31	1	47	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	32	2	47	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	34	0	41	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	35	1	46	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	36	4	44	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	38	1	43	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	40	2	42	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	41	2	44	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	42	0	44	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	44	1	41	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	45	4	40	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	46	4	41	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	47	8	47	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	47	9	47	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1
hyb	0.00	51	1	44	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	52	2	49	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1
hyb	0.00	53	7	45	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	1	-1	1

Results lie within (-1) or outside (1) the 0.05 confidence interval of the combined control scores.
*** the 821 control scores are excluded in the calculation of Efficiency Indices (see text).

APPENDIX B

Table 5. The number of progeny eclosing over a 26 day period from single **nasuta** females eclosing on Day-0.

Drosophila nasuta: **Temporal emergence pattern and fecundity.**

20 replicas																				DAY	score	SUM
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0.10	2
2	14	6	2	0	14	4	4	0	6	0	0	0	7	0	0	0	1	0	0	14	3.00	60
7	3	4	2	1	3	1	11	0	4	1	4	8	6	6	10	16	14	15	14	15	6.50	130
3	6	3	0	8	6	1	7	6	10	5	8	9	13	17	5	3	17	6	3	16	6.80	136
5	7	8	1	10	14	4	3	5	8	2	2	4	8	4	6	5	3	3	2	17	5.20	104
11	5	9	9	8	9	10	10	6	5	0	5	7	3	1	8	16	3	8	1	18	6.70	134
11	3	7	23	3	3	9	6	1	7	2	8	8	11	9	18	10	10	13	14	19	8.80	176
5	3	4	5	5	4	12	9	4	12	0	2	3	9	10	5	2	15	6	13	20	6.40	128
6	8	4	7	2	10	13	7	8	11	0	4	15	12	9	5	4	7	4	4	21	7.00	140
0	12	7	7	5	4	5	3	11	5	0	9	5	8	6	9	13	4	9	15	22	6.85	137
12	3	3	9	11	6	13	7	5	7	0	3	2	6	16	7	6	8	10	6	23	7.00	140
18	10	3	11	14	7	14	10	6	6	0	7	3	11	9	25	11	12	4	0	24	9.05	181
8	17	1	14	9	8	7	5	11	8	0	6	12	21	10	14	11	1	4	6	25	8.65	173
3	15	5	11	7	6	8	5	7	9	0	4	2	4	2	19	4	6	21	14	26	7.60	152
91	106	65	102	83	94	101	87	70	98	10	62	78	119	99	131	101	101	103	92	89.65	131	
7	8.2	4.9	7.8	6.4	7.2	7.8	6.7	5.4	7.5	0.8	4.8	6	9.2	7.6	10	7.8	7.8	7.9	7.1	6.89		

APPENDIX B

Table 6. The number of progeny eclosing over a 26 day period from single **kepulauana** females eclosing on Day-0.

Drosophila kepulauana: **Temporal emergence pattern and fecundity.**

17 replicas																	DAY	score	SUM
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17			
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0
0	0	3	0	0	1	0	0	2	0	1	5	0	0	0	0	0	14	0.71	12
0	3	14	13	0	4	0	0	5	0	7	8	6	7	0	2	2	15	4.18	71
1	4	3	6	5	10	4	5	11	1	7	5	6	11	8	4	8	16	5.82	99
10	10	4	3	6	8	19	10	13	11	3	14	7	1	6	5	8	17	8.12	138
14	8	5	4	6	6	3	7	5	7	0	0	6	4	6	8	2	18	5.35	91
3	14	8	10	3	1	2	2	8	8	7	6	7	4	10	3	10	19	6.24	106
4	5	6	9	1	7	15	6	6	8	4	1	7	9	3	4	6	20	5.94	101
6	4	9	8	0	3	3	8	9	8	9	1	4	4	11	2	7	21	5.65	96
3	11	10	7	1	6	4	2	13	6	3	1	5	1	1	4	8	22	5.06	86
10	5	4	7	2	2	7	5	6	7	3	2	5	6	2	10	3	23	5.07	86
4	3	7	7	0	2	7	11	3	6	5	7	11	12	5	4	10	24	6.12	104
3	2	8	7	0	2	2	5	6	10	6	4	3	9	3	2	8	25	4.71	80
1	2	8	5	0	2	13	3	5	4	4	6	4	9	3	9	2	26	4.71	80
59	71	89	86	24	54	79	64	92	76	59	60	71	77	58	57	74	67.65	92	
4.5	5.5	6.9	6.6	1.9	4.2	6.1	4.9	7.1	5.9	4.5	4.6	5.5	5.9	4.5	4.4	5.7	5.20		

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