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STUDIES ON THE MATING BEHAVIOUR OF SOME SPECIES OF ZYGOPTERA (ODONATA) AND *DROSOPHILA* (DIPTERA)

Hugh Mereth Robertson

A Thesis Submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, South Africa for the Degree of Doctor of Philosophy

Johannesburg 1982

Hugh M. Robertson

Minor Corrections to PhD Thesis

Page	Line	Correction
xii	4	Page number for "Reference List" is 195
7	2	But
24	14	supersedes
74	Table 15, Bottom row, R.H.S.	y^{th}
112	1	(Natal)
125	10	delete "more"
191	9	The
197		Buchholtz
205		Johnson, C. and M[illegible]. Westfall
206	2	Haskell
210		Morris
210	6	Grasse

Corrigenda

Page 42, Table 4 - Units are "log s"

Page 123, Line 5 - For "temporal region" read "temperate region"

Page 148, Lines 10, 17, 23 - For "discreet" read "discrete"

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, South Africa. It has not been submitted before for any degree in any other University.

31st March, 1982.

STUDIES ON THE MATING BEHAVIOUR OF SOME SPECIES OF ZYGOPTERA (ODONATA) AND DROSOPHILA (DIPTERA)

ROBERTSON, Hugh Mereth, Ph.D. University of the Witwatersrand, 1982.

In five separate projects, the specific-mate recognition systems (SMRS's) are described and analysed in Drosophila melanogaster and two of its sibling species (Diptera), and in various species of the genus Enallagma and the family Chlorocyphidae (Zygoptera). First, the concept of female courtship summation in D. melanogaster is questioned on the basis of unsuccessful repetitions of experiments supposedly supporting it. As a result, long courtships are considered to be an artifact of the experimental situation, involving agitation of the female. The relevance of this conclusion for sexual selection theory is discussed. Second, it is demonstrated that the mating disadvantage of yellow D. melanogaster males when courting wild-type females does not involve stimuli transmitted or received by the females' antennae, or the males' wings, foretarsi or proboscides. Involvement of the males' sexcombs cannot be excluded. Nevertheless the cause of the males' disadvantage, or the y females' receptivity to their courtship, is unclear. Third, the mating behaviour of D. mauritiana is described and analysed. It has become more light-dependent, and the males' wing vibration is more variable, than that of D. simulans, from which it apparently evolved in allopatry on Mauritius. Nevertheless its mating responses are as specific as those of D. melanogaster and D. simulans, which contradicts the view that "isolating mechanisms" should be relaxed in isolation.

Fourth, it is demonstrated that Enallagma females recognise conspecific males during tandem by the tactile stimulation of the males' anal appendages. Allopatric changes in this recognition system may incidentally result in the mechanical isolation observed in this group. Fifth, the relatively complex mating behaviour of Platycypha caligata is described and compared with that of three other chlorocyphid species. It is suggested that this complexity is related to the need for males to lead females to their defended oviposition sites, which the females examine for suitability for oviposition before mating.

It is argued that the reinforcement of "isolating mechanisms" is not generally important in speciation, and that speciation generally involves completely allopatric differentiation of SMRS's. Possible explanations of such allopatric SMRS changes are discussed using these studies as examples.

PREFACE

Courtship in animals has fascinated biologists since long before Darwin proposed his framework of evolution, in which it could be systematically studied. Subsequently, the study of courtsnip has formed much of the basis of ethology, the zoological study of animal behaviour, partly because courtship is usually a conspicuous and relatively easily studied behaviour. More recently, the study of courtship and mating behaviour in general has become of central importance to an understanding of evolution itself. This is because species, which are best defined in terms of their mating responses, are seen as important units of evolution. Thus an understanding of speciation, involving changes in mating responses, is essential. This thesis is directed at extending our understanding of the mating behaviour of certain insects, and at placing this in the evolutionary context of speciation.

My interest in animal courtship started with casual observations, made between bouts of rock-climbing, of the dramatic courtship display of the damselfly Platycypha caligata. This thesis began with a study of its complex mating behaviour. This work has been extended to include other species of both this and another family of Zygoptera. During the winters I started working on the already much studied mating behaviour of the fruit fly Drosophila melanogaster, and its two sibling species, the courtship of which I was first shown by Prof. Hugh E. H. Paterson. I naively thought this would provide an already well understood example of courtship However, as will be seen, this work has come to comprise over half of this thesis.

I am indebted to many people who contributed directly or indirectly to the completion of this work over the past four years. I thank particularly the staff and students of the Department of Zoology at the University of the Witwatersrand for the congenial and intellectually critical atmosphere in which I was fortunate to be able to study; my friends, siblings and especially my parents for their encouragement and support; and my supervisor, Prof. Hugh Paterson, for my education in evolutionary biology, constant encouragement and stimulation, and the development of what critical faculties I possess.

Fellow students, Marc Centner, Stuart Halse, Alexis Harington, Dave Lambert, Brian Levey, Judy Masters and Steve Telford, and lecturers, Craig Bielert, Robin Crewe, Neville Passmore and Hugh Paterson are particularly thanked for many long discussions. Critical comments on various parts of this thesis by Marc Centner, Judy Masters, Craig Bielert, Hugh Paterson, Jon Waage, some anonymous reviewers and especially Robin Crewe and Stuart Halse are gratefully acknowledged. Without Neville Passmore's enthusiasm and skill the sound recording apparatus used in Chapters 2-4 would never have been built. Prof. B. I. Balinsky and Dr. E. C. G. Pinhey generously gave me various specimens of Enallagma damselflies. Dr. L. Tsacas supplied the original stock of Drosophila mauritiana, and the Drosophila Stock Centre at Bowling Green State University, Ohio, supplied the y alleles. Petrus Nengwani, Bennet Selwadi and especially Lore Fourie tirelessly prepared Drosophila food bottles. The staff of the Electron Microscope Unit and the Central Graphics Service of the University of the Witwatersrand are thanked for their help. Walli Maier developed and printed many photographs,

including those in this thesis, and Dawn Durant and Mike Picker are thanked for Figures 12 and 19 respectively. Mrs. Patterson, Mrs. Rorke and Mrs. Orwin typed drafts of various sections, and Heather Merwitz typed the greater part of this final version. I was supported throughout this work by bursaries from the CSIR and the University of the Witwatersrand.

The work reported here has been submitted or accepted for publication in the following journals. Chapter 2 is in press in ANIMAL BEHAVIOUR. Chapters 3 and 4 have been submitted to GENETICAL RESEARCH and EVOLUTION respectively. Chapter 5, co-authored with H. E. H. Paterson, is in press in EVOLUTION. Chapter 6 has been published in BEHAVIOUR 79, 11-27 and ODONATOLOGICA 11, 53-58. Appendix A has been submitted to EXPERIENTIA, and the remaining Appendixes are in press in DROSOPHILA INFORMATION SERVICE.

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Chapter 1. GENERAL INTRODUCTION

1.1 Speciation

1.1a General

The diversity of life on earth is one of the major phenomena which our theory of evolution must explain. This diversity is discontinuous because it consists of an enormous number of species or kinds. While the reality of species as units of variability in this spectrum of diversity is still debated in asexual organisms and some plants (e.g. Levin 1979), in sexually reproducing animals it is undoubted. Hence a major part of our explanation of the diversity of life must involve an understanding of species and speciation.

Some time ago Mayr (1954), after elaborating his theory that speciation involves "genetic revolutions" in isolated populations, suggested that: "Rapidly evolving peripherally isolated populations may be the place of origin of many evolutionary novelties. Their isolation and comparatively small size may explain phenomena of rapid evolution and lack of documentation in the fossil record, hitherto puzzling to the paleontologist." Some paleontologists have finally become aware of this and have developed the model of punctuated equilibrium in which most evolutionary change is considered to take place during speciation, whereafter species do not change (e.g. Eldredge and Gould 1972; Stanley 1978; Vrba 1980; and see Stanley 1978 for references to earlier Russian paleontologists). Many other non-paleontologists have also argued that most evolutionary change takes place during speciation (e.g. Mayr 1963, 1970; Levin 1970; Carson 1975; Templeton 1980; Hallman 1982). Despite some negative evidence (e.g. Avise 1977; Cronin et al. 1981), the evidence

presented by the paleontologists overwhelmingly supports the punctuated equilibrium model. Recently Williamson (1981), in the first sufficiently fine-grained study of a fossil series, has documented an example of such punctuated equilibrium. This makes speciation theory of central importance to our general understanding of evolution as well as particularly the diversity of life.

Normally, before discussing speciation a species concept should be stated. Speciation theory and species concepts have, however, become interlinked, so I will use an intuitive preliminary species concept, which will be more clearly stated later. For now species are considered to be groups of individuals capable of coexisting without interbreeding with members of other species. That is, different species have mating behaviours sufficiently different to preclude interbreeding if they come into contact, and ecological requirements sufficiently different to allow coexistence without competitive exclusion. Therefore the major question to be asked is how do mating behaviours and ecological requirements change.

1.1b Sympatric Speciation

For many years various biologists, especially Mayr (e.g. 1970), have argued that such changes must generally take place in some form of allopatry, because otherwise gene flow will prevent any differentiation. Nevertheless, over the past few years various other models have been argued for, such as sympatric speciation (e.g. Maynard Smith 1966; Bush 1975), stasipatric speciation (e.g. White 1978) and allochronic speciation (Alexander and Bigelow 1960), among others. Recently, Mayr (1978), Futuyma and Mayer (1980) and Paterson (1981) have thoroughly refuted these arguments. In addition, some of the major examples supposedly demonstrating these modes of

speciation must be re-evaluated in the light of recent work. Thus Henry (1980) has reported that the Chrysopa species Tauber and Tauber (1977a,b) argued had speciated sympatrically have very different courtships which is not expected from their model. John (1981), and other authors in the same volume, have followed Key (1968) in questioning White's (1978) interpretation of the origin of chromosomal races in morabine grasshoppers as demonstrating stasipatric speciation. Harrison (1979) has found that on allozyme characters, the two species Alexander and Bigelow (1960) considered to have recently speciated allochronically are in fact rather distantly related within that group. Therefore, at least for the purposes of this thesis, only models involving at least some allopatric divergence will be considered.

1.1c Allop tric Speciation and Reinforcement

Species are usually, but not here, defined in terms of reproductive isolation which includes post-mating isolation, i.e. hybrid disadvantage, sterility or inviability, and pre-mating isolation, i.e. differences in mating behaviour which partially or completely preclude interspecific mating. It is clear that, except by invoking dubious group selection models, post-mating isolation cannot be selected for as such. Hence the genetic differences or chromosome rearrangements which cause such hybrid disadvantage or sterility must originate incidentally in allopatry, either as differential adaptation, or resulting from drift and chance fixation in small, inbred populations (particularly for certain chromosome rearrangements which immediately cause hybrid sterility). If the punctuated equilibrium model is correct and most change takes place during speciation, the prevalence of hybrid disadvantage between

closely related species indicates that considerable divergence takes place in allopatry.

Before considering the origin of pre-mating isolation, the origin of ecological differences allowing coexistence (assuming already established pre-mating isolation) will be discussed briefly Some change must take place in allopatry because otherwise there may be competitive exclusion if the populations remake contact. That not all species undergo complete divergence in allopatry before remaking contact is indicated by the frequent occurrence of interspecific competition (e.g. Diamond 1978). It has long been suggested that this competition will generate selection leading to further divergence in ecological requirements (ecological character displacement) to facilitate coexistence (see Grant 1972 for a review). However, a number of studies question this. Grant (1975) has reanalysed and rejected the major example at that time. Huey (1979) has more recently questioned whether competition will generally lead to such displacement. Hairston (1980) has also suggested that possible outcomes other than displacement or extinction of one of the populations are more likely. Slatkin (1980), in the first theoretical modelling study, has found that additional conditions to those normally considered are necessary for displacement to occur. Perhaps most importantly, Strong et al. (1979) have questioned whether ecological character displacement is generally important in the construction of communities, as is usually assumed. This has generated considerable debate (Grant and Abbott 1980; Hendrickson 1981; Strong and Simberloff 1981). Hence the importance of ecological character displacement is not well established, and the theory that communities are composed of species capable of

reasonably harmonious coexistence without subsequent changes in species characters seems generally applicable.

In a somewhat analogous fashion, changes in mating behaviour leading to pre-mating isolation may occur in allopatry, or, if hybrids are disadvantaged, may be selected for in subsequent sympatry. Argument for the first possibility is usually credited to Muller (1942) and Mayr (1970), while the second (reinforcement of isolating mechanisms) was developed by Dobzhansky (1937, 1940, 1970). Because a clear understanding of these theories and their importance is needed for this thesis, this question will be discussed in some detail.

Firstly, as noted repeatedly by many authors, these theories are not mutually exclusive. Nevertheless, they propose completely different processes which for the sake of clarity must be discussed separately. Furthermore, it is possible that one or the other is generally correct. It should be noted that, as Littlejonn (1981) correctly points out, both Muller (1942) and Mayr (1970) accepted that some reinforcement was not only possible, but probable. This position, that some divergence takes place in allopatry and this is accentuated in subsequent sympatry is probably held by most biologists. It is important to note that at least some hold that de novo reinforcement of "pre-mating isolating mechanisms" is a general model of speciation (e.g. Ayala et al. 1974; Lewontin 1974).

Secondly, divergence of mating behaviour in allopatry is at least partially independent of, or does not necessarily result from, divergence leading to post-mating isolation. Thus "species" pairs exhibiting hybrid sterility but no mating divergence are found (e.g. Koref-Santibanez 1964), many narrow hybrid zones are maintained by

hybrid sterility in the absence of mating differentiation (e.g. Woodruff 1979), and the extent of hybrid disadvantage and mating differentiation in a group of closely related species are not necessarily correlated (e.g. Carmody et al. 1962; Ahearn et al. 1974).

Thirdly, most importantly, there are at least some closely related species which produce fertile hybrids, often with no evidence of subsequent genetic breakdown in backcrosses or the F_2, yet have diverged so much in mating behaviour that they can coexist without significant interbreeding in the wild (e.g. Perdeck 1957; Mayr 1970; Lanier 1970; Liebherr and Roelofs 1975; Plunka and Potter 1977; Carson 1978, Carde et al. 1978; Byers and Hinks 1978; Wiman 1979; Kaneshiro 1980; Stratton and Uetz 1981). In such cases there can be no doubt that this divergence arose completely in allopatry, without the operation of reinforcement in subsequent sympatry.

Three lines of evidence, 1) artificial laboratory selection experiments, 2) mathematical models and computer simulations, and 3) cases of reinforcement, are usually cited to support the contention that reinforcement is at least sometimes important. These will be considered in turn.

Artificial laboratory selection experiments. Numerous selection experiments have been conducted, usually with Drosophila, in an attempt to produce divergence and reduce interbreeding between two strains or species (sympatric species which do not interbreed in the wild will often do so in the laboratory (Dobzhansky 1951)) by treating hybrids as sterile and removing all parental types which interbreed. These have often been at least partially successful (e.g. Koopman 1950; Knight et al.

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1956; Kessler 1966; Dobzhansky and Pavlovsky 1971; Dobzhansky 1972; Crossley 1974: Eoff 1977), But there have been some interesting failures. Ehrman (1971), using chromosomally intersterile strains of D. melanogaster, only found reinforcement in one of three replicates, and after more than 100 generations of selection, the isolation in this replicate had actually disappeared (Ehrman 1979). Petit et al. (1980) reported similar results using geographically separated strains of D. melanogaster, and have gathered various other reports of failures. They suggest that such experiments are only successful when species, subspecies or mutant strains which already show considerable mating divergence are used. The undifferentiated mating behaviour of geographical strains or non-mutant stocks is apparently too closely coadapted and invariable for even this strength of selection to bring about divergence. Ehrman's (1979) results remain anomalous because she used mutant strains which exhibited assortative mating at one stage (Ehrman 1971), most of which they lost during the rest of the experiment.

Paterson (1978) has pointed out, using an illustrative algebraic model, that there is a major problem with considering the successful experiments to be direct evidence for the importance of reinforcement, as many authors have done. That is, in every case the numbers of the two populations have been maintained at parity. Paterson points out that if this is not done, one of the populations will rapidly become extinct. Thus in a natural situation where a small population becomes completely sympatric with a larger population, a model presented in some textbooks, the small population would rapidly become extinct. It might be argued that frequency dependent selection or the excess reproductive replacement

capac.ty exhibited by most populations will prolong the period before extinction, but this is still not likely to allow reinforcement to occur considering that even the successful experiments above took many generations to achieve only partial divergence. Thus the only conclusion that can be drawn from such studies is that there is usually some variability for selection to operate on, but this will have little effect if some divergence has not already taken place. Even then, these experiments do not demonstrate that reinforcement will result from such selection because they do not take into account the strong possibility that extinction will occur.

Mathematical models and computer simulations. The first of these was the much quoted but never published study by Bossert (see Wilson 1965). Bossert studied a closed genetic system similar to that used in the selection experiments by maintaining the numbers of the two populations. Thus he was not modelling the race between extinction of one of the populations and reinforcement, but rather the race between reinforcement and fusion of the populations when the hybrids are less than sterile and only partially disadvantaged. His simulations are therefore as unrealistic as the selection experiments and only demonstrate that considerable hybrid disadvantage is necessary for reinforcement to be a possibility. Bossert apparently felt that his model would still work if the numbers were not maintained at parity, but did not substantiate this.

In an interesting paper at this time, Bazykin (1969) noted that, as Paterson (1978) reiterated, heterozygote disadvantage in a closed genetic system is highly unstable and leads to the elimination of one of the genotypes. Applied to secondary sympatric recontact situations this means that the smaller population will become

extinct if the hybrids are sterile, as Lewis (1961) had already demonstrated experimentally in some plants (Clarkia), or, if the hybrids are only partially disadvantaged, the cause of this disadvantage will be eliminated and again a single population will result, as Mettler (1957) and Caspari and Watson (1959) had already demonstrated. In the open genetic system situation hybrid sterility will lead to a narrow hybrid zone (e.g. Woodruff 1979; Barton 1980, 1981) and partial hybrid disadvantage will lead to a steep gradient (cline) which the genetic elements causing the disadvantage will not be able to cross, while any other compatible genotypes will cross the barrier and occur in both populations (e.g. Key 1968; Endler 1977; Moran and Shaw 1977; Paterson 1981). For reinforcement to occur, it must win the race against these processes, and this is what must be realistically modelled, because these processes are not taken into account in the verbal models usually discussed.

This point was forcefully brought home in the next study. Crosby (1970) conducted a comprehensive computer simulation study of reinforcement in flowering plants. His initial model was similar to the selection experiments or Bossert's model, but without maintenance of numbers. That is, he simply modelled the normal verbal arguments. His immediate finding was that one of the populations became extinct, even with only up to 75% hybrid disadvantage (sterility), thus confirming Lewis (1961) and Bazykin (1969). If the hybrids were more fertile, the other possible outcome, fusion, resulted. Reinforcement was not possible in a closed genetic system. Crosby then successfully conducted two runs (one with different ecological conditions) in which two initially

contiguous populations over some hundreds of generations experienced reinforcement for flowering time, leading to effective isolation and successful coexistence. A number of other points can be made from this study.

Firstly, Crosby found that the divergence in flowering period spread into the two allopatric populations so coming to characterize both populations. Moore (1957) had suggested that this was unlikely to happen, since, contrary to Dobzhansky's (1940) argument that changes in mating behaviour selected for in sympatry would be neutral in allopatry, he felt that such changes would probably impair mating in allopatry and so would not be able to spread.

Secondly, Crosby found that if there was even a slight disadvantage connected to the genetic changes leading to pre-mating isolation, reinforcement did not occur, emphasizing the weak nature of the second-order selection involved in reinforcement. Indeed Caisse and Antonovics (1978) suggest that this weakness, and not gene flow, habitat stability, stabilizing selection in coadapted systems or developmental stability, is responsible for the "integrity" of species and their apparent reality.

Thirdly, Crosby unfortunately did not examine the situation where hybrids are sterile, apparently because he considered these to be full species already. As discussed above however, the formation of a narrow hybrid zone is the most likely outcome of such a situation, and the flow of immigrants to maintain the zone would probably swamp any tendency to reinforcement, as Woodruff (1979) among others has found in nature.

Fourthly, and most important, flowering period is a fairly simple character to change, as Paterniani (1969) demonstrated in a

plant version of the Drosophila selection experiments. As these Drosophila experiments discussed earlier show, animal mating behaviours are considerably more difficult to change. This is an important problem for the generality of Crosby's model, and those discussed below. Again it must be noted that, contrary to the assertions of some authors (e.g. Sved 1981b), Paterniani's experiment does not demonstrate Crosby's model in nature because Paterniani used a closed genetic system with artificial maintenance of numbers at parity. This greater lability of flowering period (but not pollen-stamen incompatibility or pollinator systems (Levin 1970)?) together with the incidence of hybridization and partial hybrid fertility (Levin 1979) might suggest that reinforcement is theoretically more likely to be important in such plants than in animals.

Subsequently various authors have presented similar models, which substantiate and extend some of these conclusions. Anderson (1977) and Caisse and Antonovics (1978) make interesting attempts to pull together gene flow (due to partial hybrid fertility) and lack of ecological divergence leading to competition together with reinforcing selection. Both disagree with Crosby (1970) and confirm Moore's (1957) suggestion that allopatric populations will remain undifferentiated. As noted above, Caisse and Antonovics (1978) emphasize the weakness of the reinforcing selection. Anderson (1977) confirms that interbreeding with hybrid sterility leads to a narrow hybrid zone with no reinforcement. Both however still use excessively simple determination of the mating behaviours. This is also a major problem with a more recent study (Sawyer and Hartl 1981). Sved (1981a,b) has recently developed a more realistic model

in which the mating behaviour is more complicatedly determined, especially with respect to separate genetic control in either sex. Unfortunately he only considers closed genetic systems with maintenance of numbers. Even in this situation he finds that the more complex determination of the mating behaviours prevents divergence from random mating. Reinforcement was only possible if considerable divergence had already taken place. This agrees with the Drosophila selection experiments and confirms Paterson's (1978) contention that mating behaviour is difficult to change (see also Moore 1979). As already discussed, Sved's closed genetic system, like the others, cannot work in nature. Furthermore, if his realistic model of mating were used in the above models of the open genetic system where the numbers are maintained by immigration, which has a retarding effect on divergence, reinforcement will probably be found to be an unlikely outcome.

M. Centner and D. Lambert (pers. comm.) have approached this problem, taking into account all the factors and problems in these models, by determining what levels of lability of mating behaviour are necessary for reinforcement to be a feasible outcome in the race against extinction in open genetic systems and find that is about an order greater than that found in the successful Drosophila selection experiments.

The conclusion which can be drawn from these studies is that, for animals and probably most plants, reinforcement is impossible if there is no initial divergence and the selection against hybrids is not close to complete. They also confirm that closed genetic systems probably never lead to reinforcement. As Centner and Lambert show, and would probably be discovered if Sved's model was

developed to consider the race between extinction (or formation of a stable hybrid zone or cline) and reinforcement, reinforcement is unlikely in any real situation, including open genetic systems.

Cases of reinforcement. Numerous cases of reproductive character displacement (more divergence in sympatry than in allopatry) have been reported (e.g. Levin 1970; Mayr 1970; Bush 1975). Various authors have demonstrated that most of the older evidence does not satisfy the criteria for establishing a case of character displacement (e.g. Thielcke 1970; Grant 1972, Loftus-Hills 1975; Fouquette 1975). Hence only the, by now classic, case presented by Littlejohn (1965) and studies more recent than 1974 will be considered. Littlejohn's (1965) study of character displacement for mating calls between the frogs Litoria (=Hyla) ewingi and L. verreauxi in S. E. Australia (see also Littlejohn and Loftus-Hills 1968) is an excellent starting place because it illustrates a major problem with such studies. This is that the situation has subsequently been found to be considerably more complex than originally thought. There are at least three more subspecies and species, and probably other differentiated populations, involved in this area (Watson and Littlejohn 1978). Some of these exhibit considerable post-mating isolation. Since this can only have arisen in allopatry, it can be concluded that these frogs have recently spread from refuges in which they were isolated. Most of these pairs of subspecies have undifferentiated mating calls and so form narrow hybrid zones with, it should be noted, no evidence of reinforcement. In this light the original case of "reinforcement" can be re-examined. Watson and Martin (1968) found that hybrids between the sympatric, but not the allopatric or

allopatric and sympatric, populations show considerable inviability. They propose an ecological character displacement explanation of this, but it is more plausible that it originated in refuges as above. If so, why not also the divergence in the mating calls (and calling sites) which would then allow coexistence for this pair? The point here is that authors, when observing that allopatric populations or species are sometimes non-differentiated while sympatric populations are always differentiated, often assume that the differences were selected for in sympatry. This argument can be turned around however, in a similar fashion to the arguments about community formation and ecological character displacement, to say that only differentiated populations can achieve sympatry (Paterson 1978, 1981, 1982; Passmore 1981; Hailman 1982).

The other two major studies on frogs are by Fouquette (1975) and Ralin (1977). Ralin's should be treated as a prima facie case, but Fouquette's has two further problems. Firstly, in sympatry, the calls of the species which diverges (the other remains constant) become remarkably variable. The opposite is expected if selection for avoidance of mismating is operating. Secondly, his allopatric populations are already so differentiated as to make mismating unlikely in the first place. This illustrates a point of potential confusion. Character displacement is defined as divergence resulting from the presence of another species (Grant 1972). Reinforcement is actually a special case in which mismating leading to production of disadvantaged hybrids selects for the divergence. Thus Fouquette's is probably not a case of reinforcement. This same problem applies to Waage's (1979a) example in calopterygid damselflies. His, if anything (there is a possible external cause which he unconvincingly

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attempts to discount), may be an example of character displacement to avoid interference by non-conspecific males (see also Waage 1975). This is not comparable to reinforcement, as Littlejohn (1959, 1964) suggested, because the dynamics of the evolution of such displacement are quite different, i.e. there is no race against extinction if there is actually no interbreeding in the first place.

The final example to be considered is that of the fruit flies Drosophila arizonensis and D. mojavensis (Wasserman and Koepfer 1977, 1980; Markow 1981). Again there are two problems. Firstly, the hybrids are largely fertile and form hybrid swarms in the laboratory (Mettler 1957; Zouros 1981). As already discussed, and emphasized by Futuyma (1979; p 407), fusion is more likely if such randomly mating populations meet in secondary contact. It is therefore likely that the divergence observed is not reinforcement. Secondly, the present sympatric populations may, as argued above for Littlejohn's frogs, have diverged allopatrically from both each other and their allopatric populations and so be able to coexist. This explanation gains support when a related study by Zouros and d'Entremont (1980) is considered. We find that D. mojavensis has two races and that there is considerable divergence in mating behaviour between various populations of the race B. Zouros and d'Entremont (1980) suggest that this is related to reinforcement of some of these populations in the presence of D. arizonensis. However, two insular populations which are allopatric to D. arizonensis are also partially sexually isolated from the other allopatric populations. If an intraspecific explanation is needed for this, why not also for the divergence in the mainland populations of D. mojavensis and D. arizonensis?

These criticisms are designed to show that alternative explanations should still be considered for the best documented cases. Further study, particularly of the examples of Littlejohn (1965), Ralin (1977) and Wasserman and Koepfer (1977) is needed before they can be accepted as demonstrating reinforcement in nature. For example, H. C. Gerhardt is presently re-examining the Litoria ewingi complex (pers. comm.). Two final points can be made if these examples are accepted. Moore's (1957) point that any divergence selected for in sympatry will not spread into allopatry is again supported, and therefore it must be noted that these examples do not provide direct evidence for speciation by reinforcement, because the entire populations do not have the diverged mating behaviour. Speciation would only be complete if the two populations slowly became completely sympatric with reinforcement taking place at the advancing edges of each population until one or both is completely differentiated.

Finally it must be noted that there is evidence that reinforcement often does not result from secondary contact because, as discussed above, a narrow hybrid zone is formed because the hybrids are so strongly selected against (e.g. Bigelow 1965; Watson and Littlejohn 1978; Woodruff 1979; Gartside 1980; Heth and Nevo 1981). Here the immigration from the allopatric areas which maintains the hybrid zone swamps any reinforcement.

Taking all this evidence together, what circumstances might lead to speciation by reinforcement? Clearly the model of de novo reinforcement from random mating is never tenable. In a closed genetic system (complete sympatry) fusion or extinction of the smaller population are the only possible outcomes, depending on the

level of hybrid disadvantage. In an open genetic system (parapatry), fusion or the formation of a steep gradient (cline) or narrow hybrid zone will result. Only when considerable divergence has already taken place might reinforcement win the race. Even then, this is only really possible in the situation where two fairly large populations meet. But if this situation is to be taken seriously, Moore's (1957) point must be accounted for.

Clearly then, reinforcement cannot be a crucial or even generally important factor in speciation (Paterson 1978, 1982). Because there is no doubt that mating behaviour can diverge completely in complete allopatry (evidence from the interfertile species mentioned earlier and numerous examples of closely related, allopatric, diverged species (e.g. Anderson and Ehrman 1967; Mayr 1970)), this should be our general model of divergence in mating behaviour, and hence speciation. Consideration of how this divergence takes place will be deferred to the General Discussion (Chapter 7).

1.2 Species Concepts

We can now reconsider our concept of species. The generally accepted Biological Species Concept developed by Mayr (1970) and Dobzhansky (1970) states that species are composed of potentially interbreeding populations which are reproductively isolated from other such populations. Over the years some authors have raised various objections to this concept of species (e.g. Bigelow 1965; Sokal and Crovello 1970; Scudder 1974; Wiley 1974). While many of these criticisms are valid, the solutions offered have never been more satisfactory, and this concept remains intact and generally used (Littlejohn 1981).

An unfortunate consequence of the general, but as argued above unjustified, acceptance of the verbal model of reinforcement as generally important in speciation is that the concept of "isolating mechanisms" presented by Dobzhansky (1937, 1940, 1970) is used throughout the literature on evolution and behaviour. This term, whether intended or not, when used in an evolutionary context, implies that the feature to be called an isolating mechanism was selected for to function (sensu Williams 1966) in preventing interbreeding . This is clearly not so for hybrid sterility, and so some biologists have started using the term "isolating barriers" which is more neutral. Nevertheless, when applied to differences in mating behaviour, as ethological isolating mechanisms, it is often used in the sense of functioning evolutionarily to prevent interbreeding. If reinforcement is not generally important then this usage is unwarranted. Mating behaviour should rather be viewed internally, i.e. within species, and any consequences for interbreeding or lack thereof should be viewed as incidental consequences of changes in allopatry. Hence the term "isolating mechanisms" should be discarded.

This is the view presented by Paterson (1978, 1980, 1981, 1982), who suggests that species be defined as individuals which share a common specific-mate recognition system (SMRS). As an incidental result of changes in such an SMRS in an allopatric isolate, members of the new species will not mate with those of the parental species. Thus, while ethological isolation is a real phenomenon, it results from changes in SMRS's which are not selected for, in contrast to ethological isolating mechanisms. Paterson has termed his concept the Recognition Concept which is contrasted with the usual concept

called the Isolation Concept, because of the emphasis it places on reproductive isolation.

A final point is that when using the recognition concept, populations exhibiting post-mating reproductive isolation but no pre-mating isolation such as those discussed earlier, and particularly as found forming narrow hybrid zones, are not considered to be distinct species, as Bigelow (1965) and others correctly argue they should be using the isolation concept. This is fully realistic because, if reinforcement is unlikely, the only stable outcome of the meeting of such populations is a narrow hybrid zone with minimal gene exchange between the two populations (a steep cline already implies that they constitute one large population). Although these populations are reproductively isolated, they are at an evolutionary stalemate and will remain parapatric, i.e. effectively geographically isolated. They therefore best fit the definition of subspecies as geographically isolated diverged populations.

Thus our understanding of speciation depends on an understanding of how an SMRS can change in allopatry. This will be considered in the General Discussion (Chapter 7). This thesis is initially aimed one level lower, i.e. at extending our understanding of mating behaviour and SMRS's.

1.3 This Thesis

Five projects were undertaken, three on Drosophila species and two on various species of Zygoptera. These will be presented as independent studies in separate chapters. The overall purpose of each study will be described briefly here so that it can be kept in mind while reading the details in each chapter. Some of these

aspects will only be returned to in the General Discussion (Chapter 7). A number of additional minor studies are presented as Appendixes and will be referred to when appropriate.

In Chapter 2 I examine the concept of female courtship summation in Drosophila melanogaster. Throughout the literature on Drosophila courtship it is assumed that, together with specific-mate recognition, the male's courtship stimulates the female which supposedly summates the various courtship stimuli until a threshold is reached, whereupon she mates, i.e. there is female courtship summation. If true this would provide a form of "female coyness" which is important for models of sexual selection.

In Chapter 3 I follow Bastock (1956) and numerous subsequent workers in attempting to solve the problem of what has changed in the courtship of yellow mutant D. melanogaster males which causes wild-type, but not yellow, females to reject them as mating partners. Apart from interest in using this abnormality as a means of studying normal D. melanogaster courtship, this is of considerable importance in the light of the above discussion of reinforcement. It has been suggested (Dow 1976b) that the change in the mutant males' behaviour and the mutant females' receptivity to this are both part of the mutant phenotype, i.e. the genetic determinants of the mating behaviours of both sexes are the same. This would remove much of the difficulty in changing mating behaviours which was above considered to be a major retarding influence on reinforcement.

In Chapter 4 I examine the mating behaviour of the recently described sibling species of D. melanogaster and D. simulans, D. mauritiana (Tsacas and David 1974), which is isolated on the

island of Mauritius. Apart from interest in how its mating behaviour has changed during such an apparently allopatric speciation, it can be used to test a prediction from the isolation concept of species. That is, as Mayr (1970) among others has noted, such isolated species are expected to have "relaxed" ethological isolating mechanisms. In other words, their mating behaviour should be variable and non-specific when there are no sympatric closely related species with which mismating may occur.

In Chapter 5 I demonstrate that there is a tactile recognition system operating during tandem in the mating of Enallagma damselflies (Zygoptera). Apart from extending our understanding of specific-mate recognition in odonates, this may provide an alternative explanation for the mechanical isolation observed between some Enallagma species, and in some other groups of the Odonata. Furthermore this provides an analogy for Paterson's view that ethological isolation is the result of changes in SMRS's.

In Chapter 6 I describe the mating behaviour of the Zygopteran species Platycypha caligata and some of its relatives. I was particularly interested in the elaborate courtship displays exhibited by these species, and have developed a testable general theory for the sporadic evolution of such courtship displays in the Odonata.

Finally, before proceeding, the courtship of D. melanogaster must be described briefly, because some knowledge of this is assumed for Chapters 2-4. When a pair of flies are placed together in a small observation chamber, some time passes before the male taps the female with his foretarsi. He may then proceed to court her. He follows her, extending one wing at a time and vibrating it, licks her

genitalia briefly, and then swings his genitalia forward ventrally to attempt copulation. If successful he mounts her between the wings and copulation lasts approximately 20 min (see also Appendix F). If he does not succeed, he repeats the sequence, often many times, until successful. The female responds with a series of rejection responses, including wing flicking, kicking, curling of the abdomen and decamping, until she is prepared to allow intromission. There is no obvious acceptance posture by the female except perhaps sometimes a lack of movement and opening of the vaginal plates. Because the wing vibration is so prominent a part of the courtship, it has been well studied. Two sound components are produced, pulse song (consisting of sound pulses) and sine song (a tone which on an oscilloscope resembles a sine wave) (Schilcher 1976a). Other details will be given later. Bastock and Manning (1955), Manning (1965), Spieth (1968, 1974), Spiess (1970) and Burnet and Connolly (1974) provide general descriptions and reviews of Drosophila mating behaviour.

Chapter 2. FEMALE COURTSHIP SUMMATION IN *DROSOPHILA MELANOGASTER*

2.1 Introduction

Excluding functions applicable to only certain species, courtship is usually considered to have two major functions, "species recognition" and sexual stimulation of the female (e.g. Bastock 1967 ; Chapman 1970; McFarland 1981). While the importance of what is termed "species recognition" (or rather the SMRS) is generally accepted, some workers have questioned the importance of sexual stimulation (e.g. Kennedy 1966; Barrass 1976, 1979). The concept of sexual stimulation of females by stimuli from the males during courtship is particularly firmly established in the literature on *Drosophila* courtship where it has been used by most workers in the field (e.g. Merrell 1949; Mayr 1950; Spiess 1970; Burnet and Connolly 1974; Spieth 1974; Bennet-Clark et al. 1976). It is used to explain the variation in duration of courtships, and particularly long courtships, observed in the laboratory, when this is not due to variation in the quality (as regards "species recognition") of the males' courtship. Although there is no generally used description of sexual stimulation in this literature, that of Manning (1967a, p 240) is representative: "*Drosophila* courtship is best regarded as the means whereby a male provides a female with a stream of stimuli whose effects summate, finally reaching a critical level where she accepts him". Here I examine the evidence for this female courtship summation (FCS) in *Drosophila melanogaster*, the species with which the major experimental studies have been done by Ewing, Cook and Schilcher.

Ewing (1964) found that progressive removal of the males' wings led to a linearly correlated decrease in their "sexual

success", which he interpreted as demonstrating the additive nature of the stimuli provided by the males' wing vibration.

Cook (1973a) presented evidence showing that the courtship or wing vibration requirements of the summation processes of individual females are constant over four repeat measurements (matings). His results, as presented in his Table 1, unfortunately contain two simple computational errors, and one result is incorrectly presented as being statistically significant. Crossley and McDonald (1979) however recently reported similar results.

Schilcher (1976b) found that prestimulation of females with simulated sine song, but not pulse song, before mixing with wingless males led to enhanced receptivity of the females, suggesting that sine song is more important for FCS. This study supercedes that of Bennet-Clark et al. (1973).

In order to evaluate this evidence I repeated the experiments of Ewing (1964) and Cook (1973a). The results will be presented in four sections. Firstly, Cook's (1973a) repeat mating experiment was done with separate measurement of pulse and sine song. This allowed examination of Schilcher's (1976b) suggestion. In addition, as Cook (1973a) recommended, male variation over the repeat matings was eliminated in two of four replicates. Secondly, the raw data from this experiment were used to examine the prediction from the concept of FCS that more intense male courtship should lead to shorter courtship durations. Again the separate measurement of pulse and sine song allowed examination of Schilcher's (1976b) suggestion. Thirdly, these raw data were again used to examine the relationship between male latency (lag) to courtship and the subsequent courtship duration, which suggests an alternative explanation of the

variation in courtship duration observed in the laboratory. Finally, in an attempt to separate explanations based on FCS of species recognition (see Barrass 1976), Ewing's (1964) progressive wing remov ' experiment was repeated with an innovation, and using both mass and single mating techniques.

2.2 Materials and Methods

2.2a ,ies

The Canton-S strain of D. melanogaster was used. The flies were maintained by mass culture on yeasted agar-molasses-cornmeal food in half-pint milk bottles at 25 ± 1°C. A constant light regime was used so that mating tests could be done throughout the day. Virgin males and females were collected at 12 h intervals, sexed under light ether anaesthesia, and isolated by sex in 10 x 2.5 cm vials with 1-2 cm food. All transfers were made by aspirator.

2.2b Repeat Mating Experiment

Equipment. To do many matings with recordings of both pulse and sine song, a sound recording system similar to that of Crossley and McDonald (1980) was built. A condenser microphone (Calrec CB70 with a CC52 capsule) was suspended by rubber bands inside a 0.4 m square soundproof box, constructed from double-layered masonite random drilled acoustic tiles, and insulated from substrate vibrations by sheets of high density fibreglass. The protective grill of the microphone was the floor of the mating chamber. A detachable piece of clear perspex tubing with a circular coverslip glued onto it constituted the walls and ceiling, yielding a chamber 0.6 cm deep and 2.1 cm in diameter. Flies were introduced via a hole in the wall which was plugged with a rubber stopper. By carefully lifting the chamber off the microphone, copulating

couples were easily collected by aspirator. Single flies were gently shaken and aspirated out via the hole (Fig. 1).



Figure 1. The condenser microphone suspended in elastic bands with the mating chamber on top.

Access to the box was via an 18 cm diameter hole which was plugged with masonite tiles. A small hole (diameter 6 cm) in the top of the box covered by glass allowed the flies to be observed through a Wild M3 binocular microscope on a moving arm stand. The tubes of a Nachet EF 505 fibre optics cold light source were introduced via small holes in the sides of the box. This provided excellent lateral illumination of the chamber and avoided the problem of heating from the light source. The courtship sounds were recorded on a Nagra III taperecorder at a tape speed of 19 cm per s (cps), and were monitored via headphones. The entire apparatus plus experimenter were inside a temperature controlled,

sound-insulated room which was kept at 25 ± 1°C.



Figure 2. The microphone inside the soundproof box with the taperecorders and the fibre optics (left).

Procedure. For a particular replicate, the females were all collected after one 12 h interval, and kept in groups of 10 per vial. On day 2 after emergence they were housed individually in fresh numbered vials to which they were returned after each mating. Mating tests were done on days 3, 4 and 5 after emergence. Males were similarly collected from the same bottles, and kept in groups of five per vial initially and individually from day 2. In the first two replicates the males were discarded after each mating test, so that each day a new 3 day old male was paired with each female. In the second two replicates the same male was paired with a particular female each day.

For each mating, the pair of flies was gently transferred to the chamber by aspirator. Within 5 s of introduction, the chamber was plugged, a stopwatch started to record male latency to courtship, the box closed, the microscope swung into place and the taperecorder switched to "test". The tape recording was started in anticipation of courtship as the male tapped the female. The male latency was measured as the time to the first audible burst of wing vibration by the male. At this point a cumulative stopwatch was started to measure the total duration of orientation of the male to the female. This measure was used for courtship duration, instead of the total courtship duration, thus excluding breaks in courtship. Total courtship duration would nevertheless not have been much longer since breaks were few and short. Whether or not the female extruded her ovipositor in the early stages of the courtship was noted mentally and recorded afterwards. In three replicates, whether the female used another rejection response, "curling" (Connolly

and Cook 1973), i.e. pressing her genitalia against the substrate, was similarly recorded.

In successful copulation attempts the male begins spreading the female's wings within a few s of making genitalic contact, so the cumulative stopwatch and the tape recording were stopped at this point and the number of the female recorded on the tape. As soon as possible th. copulating pair was sucked up, male first, into an aspirator. Many separated immediately but others had to be sucked up against the cloth plug of the aspirator once or twice before separating. This was done as gently as possible and care was taken to ensure it was always the male which knocked against the plug. The male always released his genitalia and was not physically torn off. This method differs from those of Cook (1973a) and Crossley and McDonald (1979), but should be equally successful in preventing insemination (after which the females would not readily mate again) without damage to either sex. The flies were then returned to their respective vials, or discarded.

After each day's recordings the tapes were played back through an additional amplifier and loudspeaker at half-speed, i.e. 9.5 cps, and the amounts of pulse and sine song were simultaneously measured with cumulative stopwatches. The sum of these gave a measure of total song which was used as a presumably more accurate measure of wing vibration than the visual measures usually employed. For all the courtships in two of the replicates, the tape was again played back at 19 cps and the sequence of bursts of pulse and sine song in the first 15 s was recorded. Complex bursts of pulse and sine song were split up.

Individual females were tested at approximately the same time each day to avoid possible changes in the females' receptivity or the males' courtship intensity during the day. No such trend was in any case apparent in the results. To accommodate large sample sizes, stringent limits were placed on the male latency to courtship and the duration of courtship monitored. If courtship did not start within 5 min the flies were removed and re-introduced to the chamber, and courtship was only watched for 5 min after which the single flies were returned to their vials.

2.2c Progressive Wing Removal Experiment

Flies were kept 10 per vial, and for single pair and female choice matings the males were kept individually for 20-24 h before use. The males' wings were cut with a scalpel fashioned from a dissecting needle while under light ether anaesthesia

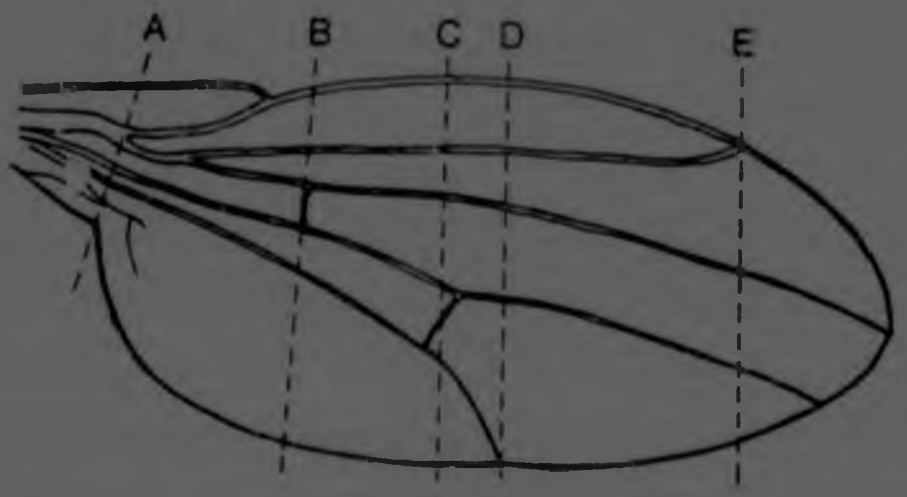

Figure 3. The five positions at which the males' wings were cut.

20-24 h before use. Their wings were cut as indicated in Fig. 3. Camera lucida drawings were made on squared paper of 20 wings in each class. Wing areas were measured in arbitrary units and

converted to percentages by comparison with the area of normal wings (Class F = 100.0%). These were A = 4.8%; B = 29.0%; C = 42.8%; D = 53.1%; E = 78.5%. In addition another class of males (Class G) had only one wing (alternately left and right) removed, yielding a wing area of 52.4%.

Three types of mating tests were used. In a form of mass mating, 10 normal females and 10 males were introduced, females first, into a 10 x 2.5 cm vial. In the first replicate the vial was empty; in the second (comparing classes D and G) it had 1 - 2 cm of yeasted food in it. The time from introduction of the females to the initiation of each copulation was recorded for 10 min. Ten vials were run per class of male per replicate.

Single pair matings were conducted by simultaneously introducing a pair of flies into a mating chamber (0.8 cm deep and 2.5 cm in diameter) moulded from perspex with a glass cover-slip lid and a perspex stopper. Only the courtship duration measured from the first bout of male wing vibration to copulation, i.e. including breaks in male orientation, was recorded. The observations were terminated after a suitable courtship duration, which is given for each experiment later (see Fig. 9).

Female choice matings were performed by simultaneously introducing a female and two males (one each of classes D and G) into the above mating chamber. These were watched until the female mated and only the class of the successful male was noted.

2.3 Results

2.3a Stability of Female Courtship Summation

In three circumstances the data collected in the repeat mating experiment were not an accurate reflection of the actual courtship. Firstly, the initiation of courtships starting

within 5 s of introduction of the flies could not be observed, so these were considered to have started at 5 s. Secondly, 16 courtships in which the female continuously extruded her ovipositor and finally laid an egg were treated as having started when the egg was laid. Thirdly, 35 (7.1%) of the 492 courtships observed did not lead to copulation in the 5 min observation period. Although 20 of these would probably never have ended in copulation (see Dow 1976a), they had to be included in the analysis and so were entered as 5 min long. Because the tape recordings of these were meaningless, arbitrary values of 60, 70 and 130 s (projected from the means for all other courtships longer than 2 min) were assigned to these courtships for pulse, sine and total song respectively.

The means of the courtship durations transformed to logarithms and taken over all replicates for each day, excluding the 35 truncated courtships and corrected for these (see Dow 1976a), decreased on day 2 and increased again on day 3 (Table 1). This suggests that the technique used for the repeat matings did not seriously affect the mating propensity of the flies.

Table 1. Means and standard deviations of courtship durations for the four replicates on each of the three days of the repeat mating experiment.

Day	Number Mating	Courtship Duration (log s)
1	152	1.5682 ± 0.5223
2	151	1.3351 ± 0.5568
3	154	1.5147 ± 0.4978

The statistical method Cook (1973a) used, i.e. Kendall's coefficient of concordance (W) (see Siegel 1956), was used to evaluate the stability or constancy of the courtship parameters for females over the three repeat measurements. This non-parametric method effectively tests whether particular females had constant courtship requirements, independent of any variation among females and the variation over the three days in the mean courtship duration.

Table 2. Concordances of courtship parameters over three repeat measurements.

Type of Experiment	N		Courtship Duration	Total Song	Pulse Song	Sine Song
		W	0.411	0.434	0.471	0.370
Female	42	x^2	50.55	53.38	57.92	45.52
Constant,		p	>0.1	>0.5	<0.05	>0.3
Male						
Varies		W	0.406	0.377	0.369	0.350
	62	x^2	74.29	68.97	67.52	64.05
		p	>0.1	>0.1	>0.2	>0.3
		W	0.450	0.355	0.320	0.405
Female	19	x^2	24.32	19.19	17.28	21.88
Constant,		p	>0.1	>0.3	>0.5	>0.2
Male						
Constant		W	0.460	0.457	0.482	0.460
	41	x^2	55.27	54.83	57.84	55.22
		p	>0.05	>0.05	>0.05	>0.05

In the repeat of Cook's (1973a) experiment, i.e. with different males for each mating, W was statistically significant at the 95% confidence level for only one parameter (pulse song) in one replicate (Table 2). Cook's suggestion that if the males were kept constant for each female the statistical significance of W should improve was clearly not supported. From Schilcher's (1976b) suggestion that sine song is more important than pulse song for FCS, more significant values of W would be predicted for sine song. This was only true for the replicate with the smallest sample size.

2.3b Importance of Courtship Intensity

The FCS concept suggests that there should be a negative relationship between the intensity of the courtship (the amount of stimulation provided by the male per unit time) and its duration. To examine this it was assumed that the repeat courtships with the same female and sometimes the same male could be treated as unique courtships. This is justified by the above finding that courtship parameters for a particular female or pair, are not generally constant.

Figure 4 is a scattergram of the relationship between courtshio duration (C) and the amount of pulse song per courtship (P/C) for all courtships except the 35 which were truncated. The well-defined hyperbolic curve (log transformation of C yields a linear regression with $r = -0.659$; $p < 0.001$; $N = 457$) shows that for courtships shorter than 55 s in duration there was a highly negative relationship. This was probably largely artifactual however, because extremely short courtships necessarily have continuous wing vibration, and as C increases so P/C inevitably decreases since the males do not vibrate continuously.



Figure 4. The relationship between courtship duration (C) and the amount of pulse song per unit time of courtship (P/C).

When courtships longer than 55 s were considered, P/C and C were not correlated ($r = -0.162$; $p > 0.05$; $N = 129$). When sine song was considered, a positive relationship, even for short courtships, was found ($r = 0.289$; $p < 0.001$; $N = 457$). This was because short courtships had relatively less sine than pulse song and long courtships more (Fig. 5 : $r = 0.477$; $p < 0.001$; $N = 457$). As a result, comparison of the proportion of total song (T/C) with courtship duration showed an intermediate pattern ($r = -0.137$; $p < 0.01$; $N = 457$), but after C = 2 s, T/C and C were not correlated ($r = -0.062$; $p > 0.05$; $N = 436$). Clearly long courtships were not the result of a lower intensity of courtship with regard to wing vibration, but the possibility that particularly intense pulse song sometimes led to short courtships cannot be excluded. Again sine song was an even less likely candidate for FCS than was pulse song.

Another measure of the intensity of courtship is the

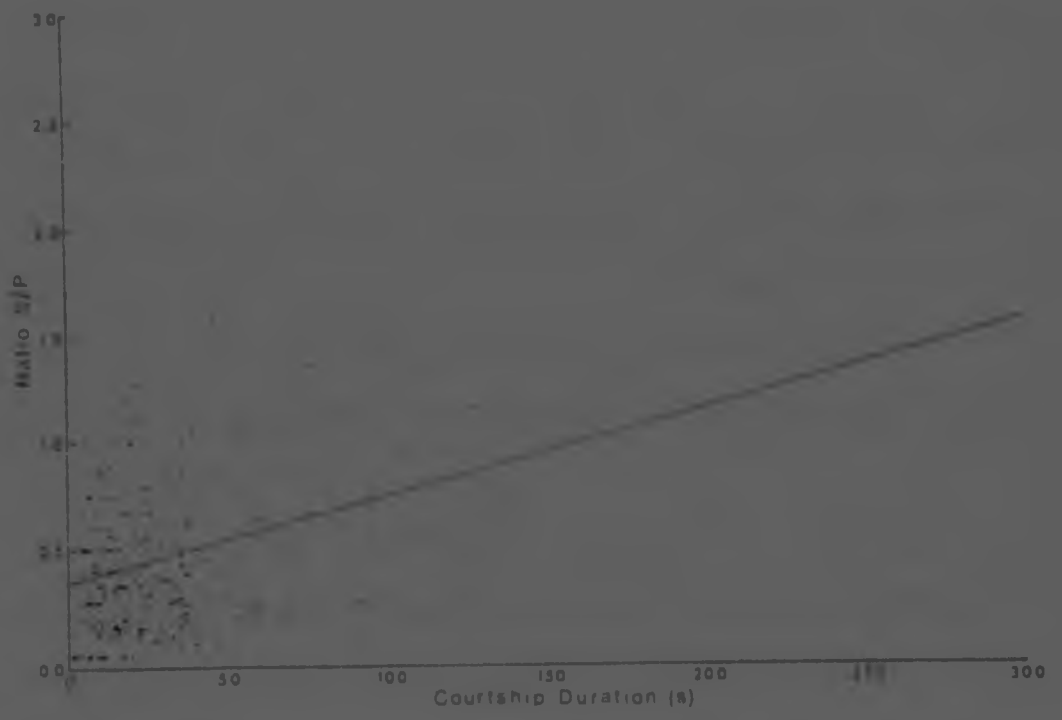

Figure 5. The relationship between courtship duration (C) and the ratio of amounts of sine to pulse song (S/P). The 35 truncated courtships are excluded. The best-fit linear regression line is plotted.

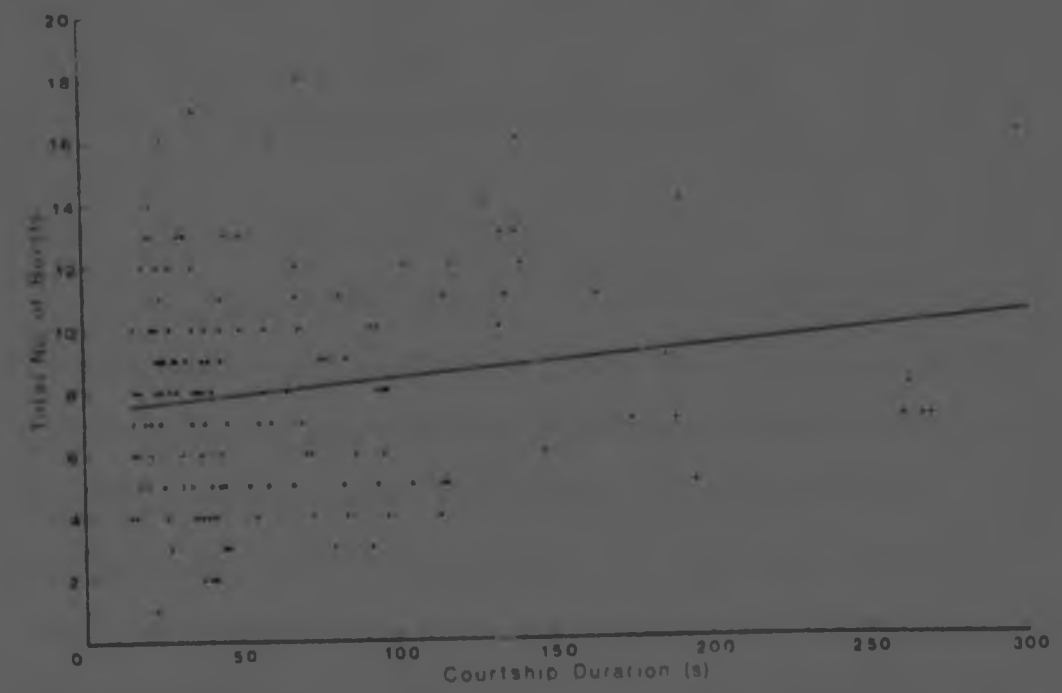

Figure 6. The relationship between courtship duration and the total number of bursts of courtship song during the first 15 s of courtship. Courtships shorter than 15 s are, of course, excluded. The best-fit linear regression line is plotted.

number of bursts of song per unit time of courtship. For 200 of the above courtships the sequence of bursts of song in the first 15 s was recorded. Of these 147 lasted longer than 15 s and so could be used to examine this prediction. Neither the total number of bursts of song nor the number of pulse or of sine song bursts was correlated with courtship duration (Table 3; Fig 6). Again longer courtships did not appear to result from less intense male courtship.

Table 3. Correlation analysis of the relationship between courtship duration and the number of bursts of courtship song.

Song	r	N	p
Total	0.162	147	> 0.05
Pulse	0.161	147	> 0.05
Sine	0.116	147	> 0.05

2.3c An Alternative Explanation

During this experiment, I noticed that long courtships often followed very short male latencies. The observations collected during the repeat mating experiment were therefore analysed to examine this possibility. As a preliminary analysis two-tailed student t-tests were used to compare the courtship durations (transformed to logarithms and excluding those truncated, i.e. extremely long courtships) on either side of various cut-off points on the male latency variable. For example, courtship durations were statistically

significantly longer when they followed male latencies which were shorter than or equal to 20 s than when the male latencies were longer than 20 s ($t = 4.41$; $p < 0.0001$), and similarly with the cut-off point at male latency = 50 s ($t = 5.16$; $p <$ [illegible]).

Plotting courtship duration against male latency revealed a strongly hyperbolic relationship (Fig. 7). The association of long courtships with short latencies and vice versa is clear. Logarithmic transformation of both axes yields a linear relationship with a highly significant correlation coefficient ($r = -0.248$; $p < 0.0001$; $N = 492$). Similarly, against male latency, total song and pulse song had correlation coefficients of -0.263 and -0.301 respectively, which indicates that pulse song yields the best fit to the hyperbolic relationship. The amount of sine song could not properly be included in this analysis since 35 (7.1%) of the courtships (all of which were very brief) did not contain any sine song (the log transformation could therefore not be used), but it exhibited a similar but less pronounced hyperbolic relationship.

The reason for short male latencies leading to longer courtships appeared to be that the female was still agitated after entry into the experimental chamber. This will be discussed later, but several additional points must be made here.

Firstly, this agitation would be exhibited largely as locomotory activity by the fly and would be comparable to Connolly's (1967) "reactivity". This activity is well known to decrease hyperbolically to a more or less stable level after about 60 s (e.g. Connolly 1967; Burnet and Wilson 1980). Thus male latency is a general indirect and inverse measure of the female's

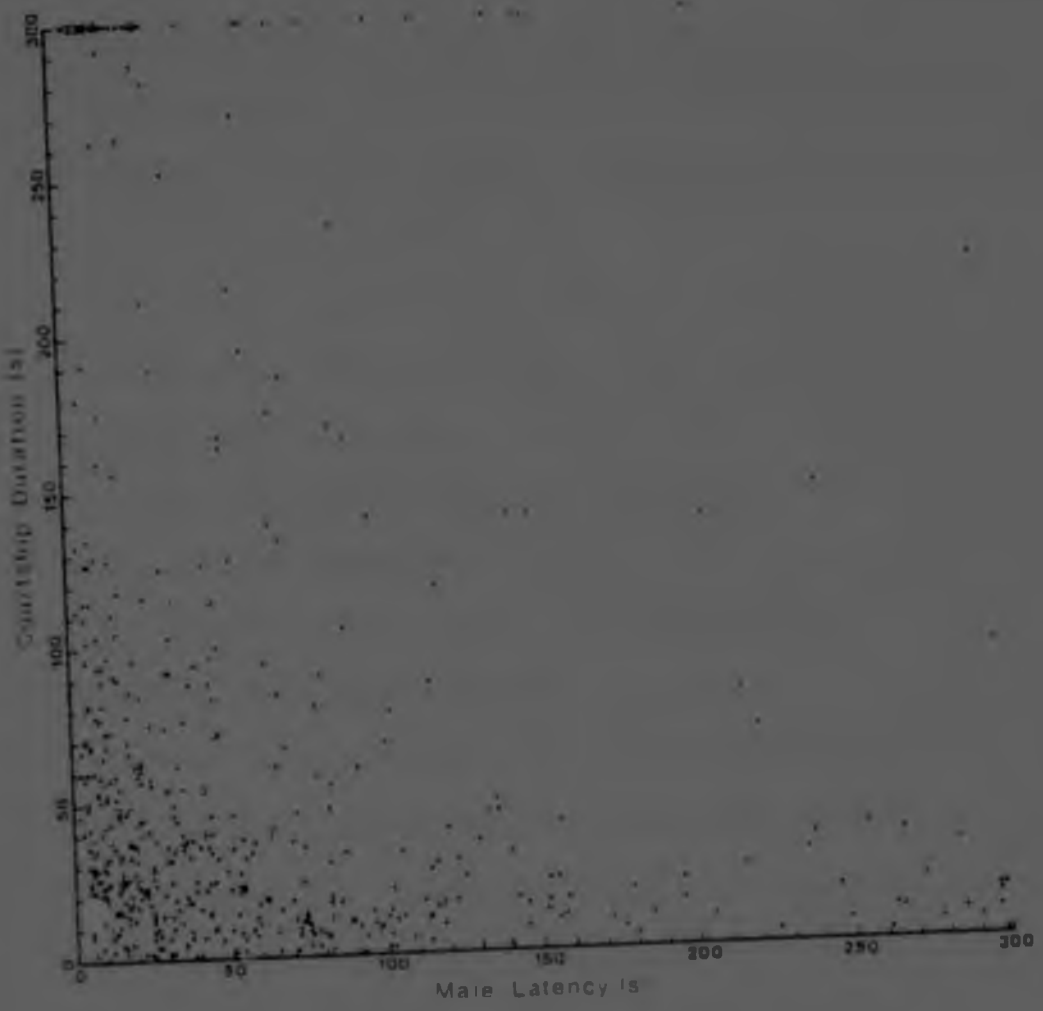

Figure 7. The relationship between male latency to courtship and the subsequent courtship duration. The 35 truncated courtships are included.

agitation. It is however not particularly accurate. For example, some females were only mildly agitated upon introduction to the mating chamber, and stood still and preened until the male found them and then mated immediately. So some of the short male latency, short duration courtships can be attributed to this. Conversely, some females had not quietened down even after 60 s. A more accurate measure of agitation, perhaps involving time

spent in and number of bouts of running and preening, should show a better correlation with subsequent courtship duration.

Secondly, courtship duration is not necessarily the most accurate measure for describing the content of a courtship. For example, sometimes a male starting to court after a long latency would then stand watching the stationary female for a long period and finally give a burst of pulse song and promptly copulate. Conversely, some males which started courting after a short latency gave almost continuous wing vibration, which was mostly pulse song, until copulation. In either of these cases the amount of pulse song would show a better fit to the hyperbolic relationship, than would courtship duration, as was found above.

Thirdly, there appeared to be an additional cause of very long courtships, which involved the interaction between the male and female. Whereas male latency is usually only dependent on the male (the female is usually passive, but sometimes actively avoids the male), once the courtship starts the male continually pesters the female. This often appeared to make the female even more agitated, and if the male's first few attempts at copulation were unsuccessful, she would press the tip of her abdomen, i.e. her genitalia, against the substrate, thus effectively preventing further attempts at copulation. This "curling" rejection response has been noticed previously (see Connolly and Cook 1973), but not connected with very long courtships. Curling was observed in 33 (8.9%) of 369 courtships (three of the above replicates) in which its occurrence was watched for. Sixteen of these 33 courtships were eventually truncated and these appeared to result directly from this female

response (only three of the other 236 courtships were truncated). The remaining 17, which ended in copulation, were all particularly long courtships (mean of courtship durations = 2.0650 ± 0.3287 (S.D.) log s, which is far longer than the overall mean for all courtships (1.4764 log s) and near the 5 min time limit (2.4771 log s)). These were eventually successful because the male temporarily became separated from the female, and on rediscovering her succeeded in copulating because she had relaxed in the interim. It is possible that the females then treated these as "new" courtships. When the 35 truncated, i.e. very long courtships, are removed from the above analysis of the relationship with male latency, the correlation coefficients improve to -0.259, -0.281 and -0.326 for courtship duration, total song and pulse song respectively. That is, they do not fit into the hyperbolic pattern exhibited by the rest of the courtships, and so need this additional explanation.

Finally, extrusion of the ovipositor is the rejection response used by fertilized females. Connolly and Cook (1973) found that extrusion constituted only 2% of the rejection responses of virgin females. The temporal patterning of extrusion by virgin females has however not been reported on. I noticed that extrusion always took place at the start of the courtship, usually in response to the first burst of wing vibration. The 16 females which extruded continuously and finally laid an egg are of course excluded. Very few virgin females extruded more than once, and it did not seem to be a determined attempt at this form of rejection. The males were not inhibited by it, as they are by the continuous extrusion of fertilized females. It

seemed that this was just the initial response of an agitated female. When the present data were analysed for the presence or absence of extrusion at the start of a courtship, it was found that extrusion followed shorter male latencies and led to longer courtship durations (Table 4), which fits this explanation, if, as argued here, females are more agitated after shorter male latencies.

Table 4. Male latency and courtship duration of courtships with or without extrusion of the ovipositor by the female.

	With Extrusion		Without Extrusion		
	N	Mean ± S.D.	N	Mean ± S.D.	p
Latency	334	1.38 ± 0.44	142	1.82 ± 0.43	<0.001
Duration	301	1.58 ± 0.39	141	1.13 ± 0.56	<0.001

2.3d Progressive Wing Removal Experiment

As Barrass (1976) suggested, and as will be argued here, there is an alternative explanation for the results of Ewing's (1964) progressive wing removal experiment. That is, partial wing removal reduces the volume of sound generated, making it harder for the female to perceive and recognise the signal being transmitted, so that she must sample more courtship before mating. In the extreme case of complete wing removal, she must eventually be prepared to mate without this signal. In an attempt to provide a definitive test of these two explanations, males with only one wing removed were included in repeats of Ewing's (1964) experiment. Since the males vibrate only one

wing at a time, and over time use them equally, from the FCS concept one would predict that these males should be accepted by the females only as readily as males with approximately half of each wing removed. If instead the females have difficulty recognising the signal, then when these males use their intact wing the signal should be readily recognisable and they might be accepted more readily than males with half of each wing removed.

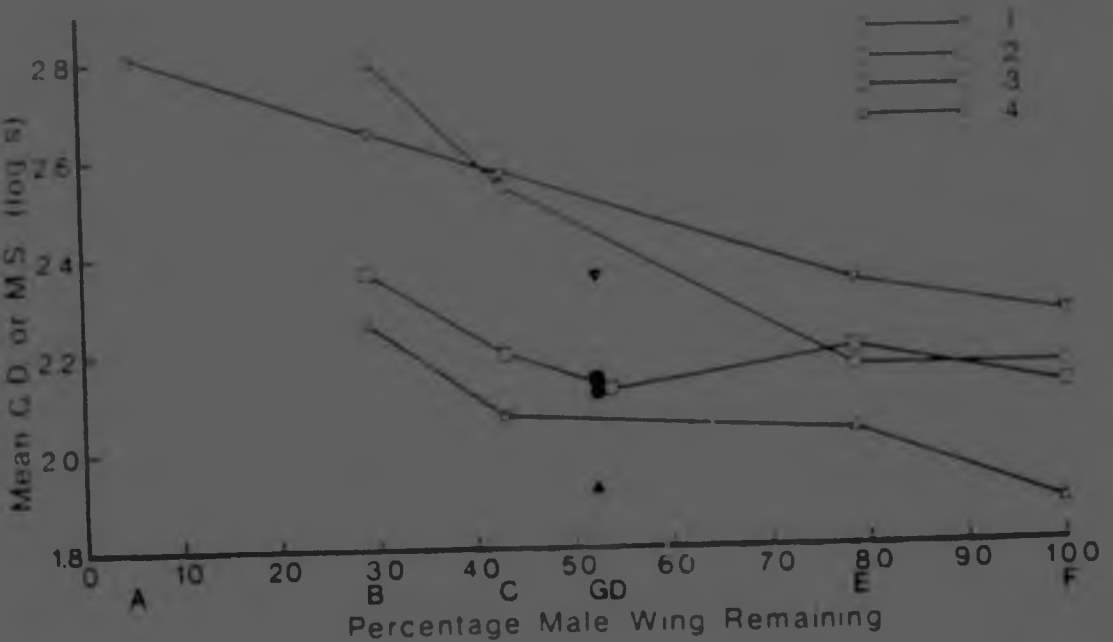

Figure 8. The relationship between percentage male wing remaining and mean mating speeds for mass-matings (1) or mean courtship durations in single-pair matings (2, 3 and 4). The data were transformed to logarithms and the means were not corrected for the truncated courtships. The solid symbols are for class G males which had one wing removed. In single-pair matings replicates 2 and 3, class A males were tested, but so few mated that means could not realistically be computed (these would have been far off the scale of the courtship duration axis). In single-pair mating replicate 2, N was 20 for each class and

observations were truncated at A 60 min, B 45 min, C 30 min, E 20 min, F 10 min and G 20 min. In replicate 3, N was 30 for classes A, B, E and F and 40 for C, D and G, and all classes were truncated after 30 min. In replicate 4, N was 20 for each class and they were truncated at B 30 min, C 15 min, E, F and G 10 min.

Figure 8 presents the results of four such experiments. In three the males with one wing were more readily accepted. The differences are not great and are difficult to test statistically. Males of class D (53.1% remaining) and class G (52.4% remaining; one wing removed) were therefore compared directly to allow a statistical test of the results. In a mass mating test, 83 out of a possible 100 class D males mated with a mean mating speed of 2.4918 $\pm$ 0.1642 (S.D.) log s, compared with 88 class G males with a mean of 2.4503 $\pm$ 0.1578 log s. In a female choice test, class G males mated in 28 of 50 trials. Again the trend is towards class G males being more readily accepted, but none of these differences is statistically significant.

The major problem with this experiment was that the class D males were only slightly disadvantaged compared to normal males (class F) (see Fig. 8), so class G males could hardly show a considerable improvement relative to them. The use of a strain showing a greater difference between class D and normal males, and/or a more sensitive method of measuring female receptivity should reveal whether there are significant differences between class D and class G males. An additional problem is that the proximal half of a wing may produce less than half the normal signal strength so that the class D males

had a disproportionately reduced overall signal strength. It might be possible to correct this bias by making careful measurements of sound pressure levels.

It is also worth noting that whereas the FCS concept suggests that one should find the sort of linear relationship Ewing (1964) reported, the hypothesis presented here suggests a sigmoid curve with initial and final plateaus. This is because initially, having some wing remaining may not raise the signal strength above the females' sensory threshhold, and finally, increase in wing area may not enhance the females' ability to hear the signal. From Fig. 8 it is clear that all four mating experiments did not yield linear relationships, but hyperbolics (the data are presented in logarithms), i.e. there is a final plateau. This hyperbolic relationship is also found if the percentage copulating after a certain time is used as the measure of mating success. If more data were collected in the initial section (0 - 15% of wing remaining) the plateau predicted might be found.

2.4 Discussion

2.4a Female Courtship Summation

Two of the three major experimental studies supporting the concept of FCS in D. melanogaster have been repeated, a general prediction from this concept has been tested, and two predictions from the third experimental study have been tested. These will be discussed in turn, and then an alternative explanation of the variation in courtship durations will be presented.

The repeat of Cook's (1973a) repeat mating experiment showed little constancy in the amount of courtship stimuli a particular

female required before mating. Removal of the male variation did not improve th s level of constancy, which suggests that the variation observed in courtship durations and other courtship parameters was not due to variation in the quantity or quality of the stimuli in the males' courtship. These results differ from those of Cook (1973a) and Crossley and McDonald (1979) in that significant levels of constancy were only found when the pulse song component of the males' wing vibration was examined separately. The parameters they examined, i.e. duration of courtship and wing vibration (total song) were not significantly constant. In the light of the errors in Cook's (1973a) paper, the more accurate measurement of courtship duration (excluding breaks in courtship) and the wing vibration parameters (audibly at half speed rather than visually) used here, and the removal of male variation used here, the present results are considered to be more reliable. Comparison with Crossley and McDonald's (1979) results is more difficult because they used a substantially different method and statistical test. Nevertheless, their levels of significance are only slightly higher than those reported here. That females should hav constant courtship requirements is a major prediction from the FCS concept. Taken together, the evidence from these three studies does not convincingly support it. It therefore can at best only be a minor explanation of the variation in courtship du ations.

The modified repeat of Ewing's (1964) progressive wing removal experiment did not yield the definitive test of the FCS concept that was hoped for, but this method may yet provide such a test. The results nevertheless did not yield the linear relationship between "mating success" and male wing area Ewing

found. This provides tentative support for the alternative hypothesis that females have difficulty perceiving the attenuated signals provided by partially wingless males.

The prediction from the FCS concept that there should be a negative relationship between the intensity of the courtship and its duration was largely unsupported. However, the possibility that very intense pulse song may facilitate short courtships could not be excluded. Recently Markow and Hanson (1981) similarly found that courtship duration was not related to the bout rate of various behaviours, i.e. the intensity of courtship. Kyriacou et al. (1978) did however find that increased intensity of courtship song improved male competitive mating ability.

Schilcher's (1976b) suggestion that sine song is more important than pulse song for FCS was not supported because, relative to pulse song, sine song was even less constant over repeat matings, and its intensity even less negatively related to courtship duration.

To conclude, the evidence for the female courtship summation concept is not convincing. In particular, differences in female requirements for courtship stimulation, and differences in the intensity of male courtship at best provide only a minor explanation of the variation in courtship durations observed in the laboratory. Clearly another explanation of this variation is needed.

2.4b An Alternative Explanation

In this study the observation that short male latencies led to longer courtship durations than did long male latencies seemed to provide an alternative explanation, based on the

agitation of the female after entering the experimental chamber. Such an effect has long been recognised for the male latency itself (Manning 1961). Upon entering the chamber both flies react to both the transfer and the new environment by running wildly around it, apparently attempting to escape. That is, the flies are agitated. When they meet, the male does not always immediately start courting the female. Indeed this usually only happens if they do not meet until after a few minutes, by which time they have calmed down considerably. If they meet very soon after entering the chamber the male may not even respond by tapping the female with his foretarsi (the usual initial male action). On meeting repeatedly he will eventually tap her, but may still not immediately respond by courting her. Thus, in addition to stochastic factors such as when and how often the flies meet (and sometimes they actively avoid each other), male latency also depends on, in the words of Manning (1961), "... the speed with which the fly can make the change over from activity responses to sexual ones". This speed, as already described, will in turn depend on how long it is before the flies meet, because the strength of the activity responses, or the agitation, of the male decreases with time.

It has always been assumed that once the male starts courting the female, she receives and summates the courtship stimuli. The possibility that when the male starts courting after a short male latency, the female is still agitated and so takes some time to change over to sexual responses, has never been seriously considered. The results presented here are interpreted as supporting this as an explanation of longer than average

courtships. Thus a female latency period, similar and subsequent to the male latency, must be recognised. This female latency will be inversely related to the female's agitation and so indirectly related to the preceding male latency. It will comprise most of the courtship duration.

It is possible that if this female latency were taken into account, an extra period of courtship would remain which would require explanation via a concept of FCS. This will be difficult to examine because the end of the female latency cannot be observed. However, because many courtships were extremely short (< 5 s) it is suggested that, subsequent to the female latency, the female mates immediately upon recognising the male as conspecific.

Although the correlation coefficient for the relationship between male latency and courtship duration is highly significant (the observation on which the above explanation is based), it is so low as to provide little predictive power ($r^2 = 0.06$), and the scatter of points in Fig. 7 is large. As pointed out in the Results, this situation is improved when more appropriate measures of the contents of the courtships, such as the amount of pulse song, are used. Similarly, a more accurate measure of the female's agitation than that provided by male latency (which at best is an indirect measure via time) should considerably improve the predictive power of this explanation.

Also, the female latency is not as simple as the male latency because the male is persistently and often excitedly courting the female. In the most simple view, one might consider the female's agitation to continue decreasing as if the male were not there until it is low enough for the change over to sexual responses to occur. This must partially be true, but

the female, as evidenced by her making rejection responses, is aware of the male's presence, and the male's courtship often appeared to have one of two, quite opposite, effects.

Firstly, some females responded to particularly intense pulse song after a short male latency by slowing down. This allowed the male to make full copulation attempts which were sometimes successful. Here the courtship appeared to calm the female, thus reducing the female latency. The possibility considered earlier that some courtships with intense pulse song were short can be re-interpreted in this light. Similar observations of courtship stimuli reducing female activity levels have been made by Bennet-Clark and Ewing (1967), Cook (1973b) and Schilcher (1976a). This would account for some more of the short male latency, short duration courtships.

Secondly, as noted in the Results, the male's courtship sometimes appeared to aggravate the female's agitation so much that she would react with the "curling" rejection response (Connolly and Cook 1973), and not mate during that courtship. Here the female latency is extended with the female never changing over. This seemed to be the explanation of most long courtships. Spiess (1980) has made a similar observation. That this, as with the first point, is an explanation additional to that involving the basic female latency can be shown by removing the 35 truncated, i.e. very long, courtships from the data relating male latency to courtship duration. As noted in the Results, this improves the correlation coefficients, thus improving the predictive power of the original explanation.

There is one more possible source of variation. The Canton-S strain used is not fully isogenic, and as discussed earlier,

females do contribute slightly to the variance in courtship duration. This can be re-interpreted now as reflecting differences in females' abilities to change over from agitation to sexual responses.

To conclude, there is a female latency period subsequent to the male latency during which the female is too agitated to change over to sexual responses. This comprises most of the courtship duration. Since this is a product of the experimental situation, the variation in courtship durations, and particularly long courtships, is considered to be artifactual. The concept of female courtship summation, which supposedly explains this variation and particularly the long courtships is therefore unnecessary.

2.4c Additional Considerations

As is to be expected when a new explanation is being advanced, little support for this idea of a female latency can be found in the literature, but there is one interesting study. Cook (1973b, c) selected for increased receptivity of normal females to courtship by wingless males, by selecting for shorter courtship durations in single-pair matings. A major change in the selected line was that the average male latency was considerably increased. That this resulted from selection only on courtship duration strongly supports the explanation advanced here that there is an inverse causal relationship between courtship duration, i.e. female latency, and the preceding male latency.

If this explanation is correct, any changes or differences in female receptivity or overall mating speed must be explained in terms of the male and female latencies and their interaction, or the quality of the courtship in terms of how readily it is

recognised by potential mates. Because the concept of FCS is used throughout the Drosophila literature, the application of this conclusion to every reported study cannot be considered here, but three examples will be presented.

As Manning (1961) noted, differences in activity levels of selected lines may influence the abilities of males and females to change over to sexual responses. For example, Burnet and Connolly (1974) found that courtship by inactive males (a line selected for inactivity which had appropriately short male latencies) of active females seldom led to copulation. This can be interpreted as resulting from the combination of the females' own difficulty in changing over and the brief male latencies, which together would seldom ever allow the females to change. over.

Differences between strains may be similarly explained. For example, re-analysis of data in Connolly et al. (1974) shows that Novosibirsk males, exhibiting latencies over twice as long as Pacific males, were three times as successful as Pacific males when courting Pacific females. Presumably, with Novosibirsk males Pacific females have far shorter female latencies.

Cook (1973a) demonstrated that immature females gradually become fully receptive, as shown by decreasing courtship durations, between 24 and 48 h after eclosion. He interpreted this as showing a gradually decreasing requirement for courtship stimuli for FCS. I have briefly repeated this experiment and confirmed his results (Appendix C). This may however, reflect a decrease in the female latency, analogous to that found by Eastwood and Burnet (1977) for male latency in immature males.

For future experimental work it would be useful to be able

to eliminate the female latency, since it appears to be artifact of the experimental situation. An initial attempt to do this by allowing the females in single-pair matings 5 min to calm down before introducing the male was only partially successful (a two-tailed t-test on the courtship durations transformed to logarithms, comparing this with simultaneous introduction, yielded $t = 2.96$; $p < 0.01$; $N = 24$ each). This was because the introduction of the male, no matter how gentle, and his initial agitated running disturbed these females again. Calming the male in a similar fashion before mixing by removing a partition in the chamber may work. Indeed this would provide a good test of the ideas presented here. Furthermore, experiments comparing solid and gauze partitions would test the possibility that during long male latencies the females are being conditioned olfactorily by the males' scent (J. McDonald pers. comm.; and see Hall et al. 1980).

Finally, since the variation in courtship durations, and especially long courtships, appears to be artifactual in the laboratory studies, one must consider what happens in the wild. From Spieth's (1974) brief description it appears that there is no male latency. Virgin females would be feeding when courted so there may be a brief female latency involving the change over to sexual behaviour, but this would be far shorter than that of an agitated female which would only be interested in escape. Short courtship durations are therefore predicted in the wild. A brief attempt to observe matings in the wild was unsuccessful, and the complete lack of such studies in the literature illustrates the difficulty involved. However, J. McDonald (pers. comm.) has made some observations in which the courtship

durations to successful copulations were all less than 15 s long. The question of the biological significance of long courtships will only merit serious study once they are observed in the wild.

Chapter 3. MATING BEHAVIOUR OF *YELLOW* MUTANTS OF *DROSOPHILA MELANOGASTER*

3.1 Introduction

Many authors have observed that *Drosophila melanogaster* males hemizygous for the *yellow* mutation are at a mating disadvantage when paired with wild-type females, but not when with homozygous *yellow* females (e.g. Sturtevant 1915; Bastock 1956; Barker 1962; Merrell 1949; Threlkeld et al. 1974; Dow 1976b; Wilson et al. 1976; Oakeshott and Hayman 1979). This phenomenon is always cited in discussions or reviews as an example of single-gene effects in behavioural genetics, however, despite considerable interest in these behavioural effects of this mutation, they have yet to be explained.

With regard to the males' disadvantage, Bastock (1956) and more recently Oakeshott and Hayman (1979) included the possibility that the changed colouration or any other visual aspect of the mutant males' courtship is discriminated against by wild-type females since the males' disadvantage remains in the absence of light. Similarly, removal of the females' antennae does not prevent this discrimination (Mayr 1950; Bastock 1956), suggesting that possible changes in either the acoustical or olfactory stimuli received by this organ are not involved.

Bastock (1956) suggested that the mutation caused a general lowering of the males' sexual motivation leading to a lower level of sexual stimulation, especially with regard to the males' wing vibration, being delivered to the females. Burnet and Connolly (1974) found that the males' activity levels are lowered and suggested that both this and the apparent lowered sexual motivation might result from lowered catecholamire levels because the biochemical pathways for pigment and neural transmitter

production are linked. However, neither of these lowered activity or motivation levels appears to explain the males' disadvantage, because Wilson et al. (1976) found that on a Novosibirsk genetic background the mutant males' courtship was no less intense than that of wild-type males, yet they were still disadvantaged. Similarly, Dow (1976c) found that increased intensity of y^1 males' courtship did not necessarily lessen their disadvantage. An explanation in terms of lowered sexual stimulation is also doubted in the light of the argument presented in Chapter 2.

Wilson et al. (1976) also suggested that the yellow pigment in the cuticle of some structures, e.g. the sexcombs, may be responsible. By examining various type-2 alleles which only affect particular structures (Nash and Yarkin 1974; Nash 1976), Burnet and Wilson (1980) eliminated this possibility. By removal of the sexcombs they found that, at least for the type-2 allele y^{b1}, these are not the problem. Finally, they observed that the mutant males have difficulty completing attempts at copulation, i.e. they do not usually make genitalic contact, suggesting that the problem lies in the transition from licking to copulation.

With regard to the mutant females' receptivity to the mutant males' courtship, Bastock (1956) suggested that it was either due to a generally increased receptivity or because they required less wing vibration stimuli and more of other stimuli. She also found that wild-type females with mutant genetic background were receptive to mutant courtship, suggesting that the mutant females' receptivity is not part of the yellow phenotype but was selected for and is controlled by various other genes. Careful scrutiny of her results, however, shows that the change found may simply be the result of changes in the receptivity of

control females, ample evidence of which is found in the rest of her paper. Nevertheless the idea is an important possible explanation. This result and explanation has been questioned by Dow (1976b), who was unable to repeat her results, and also changed the mutant females' genetic background to wild-type and found that the outbred females were still receptive. Oakeshott and Hayman (1979) have recently obtained similar results.

I report here independent data on all the above points, and especially the possible involvement of the males' sexcombs and/or proboscides in their apparently impaired attempts at copulation.

3.2 Materials and Methods

3.2a Flies

The Canton-S strain of D. melanogaster was again used as the wild-type stock. The yellow stocks were the type-1 mutants (showing complete expression) y^1 and y^{18cH1}, and the type-2 mutants (showing pattern mosaicism) y^2, y^{25}, y^{3d}, y^{bg}, y^{td} and y^{v2}.

Following Bastock's (1956) method, wild-type (y^+) flies were outbred to y^1 flies to create a stock (y^{+o}; o-outbred) with largely y^1 genetic background. Purebreeding y^{+o} stocks were extracted after 20 and 34 generations, i.e. 10 and 17 effective outbreeding generations respectively. Following Dow's (1976b) other method, y^1 and y^2 flies were outbred to y^+ flies for 20 generations (10 effective) to create stocks (y^{1o} and y^{2o}) with largely wild-type genetic background. Unfortunately the y^{1o} stock was later lost, so for the final experiments with removal of the males' foretarsi, sexcombs and proboscides another 12 generation y^{1o} stock was developed.

The stocks were maintained as before (2.2a). Virgins were collected at 12 h intervals, separated by sex under light ether

anaesthesia, kept in groups of 10-20 in 12 by 2.5 cm food vials and, except where stated, used on their third day after eclosion. All transfers were by aspirator.

3.2b Experiments

The inbred and outbred stocks (20 generations) were compared pairwise in male-choice (12 males, and 9 females of each stock), female-choice (12 females, and 8 males of each stock) and multiple-choice tests (8 pairs of each stock). These were conducted in a perspex chamber similar to that of Elens and Wattiaux (1964) which was 1 cm deep by 8 - 9 cm in diameter with a floor of filter paper containing a few drops of yeast suspension. The phenotypes of the first 8 (male- and female-choice) or 10 (multiple-choice) pairs to mate were noted by continuous observation. For the χ^{+o} 34 generations stock a no-choice mass-mating method was used. Ten females and five males were placed in a food vial for a period of 2 h, after which the females were dissected and checked for insemination.

The latter method was used for a series of experiments analysing the mating behaviour of flies with various structures surgically removed. For early experiments, the surgery was performed under ether anaesthesia, 20-24 h before use. Wings were removed with a fine scalpel, antennae were pulled off with fine forceps, and aristae and foretarsi (plus or minus sexcombs) were snipped off with fine scissors fashioned from watchmakers forceps. Experimental periods were 1 or 2 h (10 females and 6 males), or 24 or 48 h (10 females and 5 males). In later experiments, foretarsi plus sexcombs and probiscides were snipped off males which were immediately placed with the females for periods of 2 or 24 h (10 females and 5 males). For these later experiments the control males were also etherized

before mixing.

Two experiments comparing the various alleles were conducted. Firstly, in female-choice tests 12 $\underline{y}^{+}$ females, 8 $\underline{y}^{+}$ males and 8 $\underline{y}$ males were placed in a food vial. The first eight copulating pairs were aspirated after the eighth had copulated and anaesthetized to allow careful identification of the male phenotypes (especially for $\underline{y}^{bg}$ and $\underline{y}^{v2}$). Secondly, single-pair no-choice tests were conducted as before (2.2c) in the small perspex observation chambers. Only the courtship duration was measured. Observations were terminated if the pair had not copulated by 30 min. For these single-pair tests the males were kept singly for 20-24 h before use.

Detailed observations of such single-pair courtships were made via a Wild M3 binocular microscope. Sound recordings were made of $\underline{y}^{1}$ and $\underline{y}^{+}$ males courting $\underline{y}^{+}$ females with the condenser microphone equipment described earlier (2.2b). They were analysed on an oscilloscope by measuring the interpulse interval for pulse song and the sound frequency for sine song. The means of the means for each sound burst were computed for courtship by five males of each type.

3.3 Results

3.3a Effects of Outbreeding

Tables 5, 6 and 7 present the results of the multiple-, female- and male-choice tests on the stocks outbred for 20 generations. Outbreeding the $\underline{y}^{2}$ flies to wild-type effectively removed the partial disadvantage the inbred males had, and nearly restored random mating. The multiple-choice tests indicate that neither outbreeding regime had much effect on the $\underline{y}^{1}$ flies. The female- and male-choice tests allow separation of changes

Table 5. Comparison of inbred and outbred stocks by multiple-choice tests. The number of copulations in the four possible combinations are presented. Divergence from random mating was tested by chi-square.

Stocks compared	No. of Trials	$+♀-+♂$	$y♀-+♂$	$y♀-y♂$	$+♀-y♂$	p
$y^+ ; y^1$	20	83	40	68	9	< 0.001
$y^+ ; y^2$	20	78	58	29	35	< 0.001
$y^+ ; y^{2o}$	10	37	22	17	24	< 0.05
$y^+ ; y^{1o}$	10	45	22	31	2	< 0.001
$y^{+o} ; y^1$	10	46	21	30	3	< 0.001

in the sexes. There was very little change in the y^{1o} males, except that they appeared to be even less competent, and y^{+o} males did not have the mutants' disadvantage. Although in the female-choice tests the receptivity of the y^{1o} females to mutant courtship was reduced, they were still far more receptive to y^1 males than were y^+ females, and this was confirmed by the male-choice tests. On the other hand, y^{+o} females appeared in the female-choice tests to have increased receptivity to mutant courtship ($\chi^2 = 14.46$; $p < 0.001$). This was confirmed by no-choice, mass-mating tests after 34 generations of outbreeding in which y^1 males courting y^{+o} females achieved 70 of 200 possible matings compared with only 37 courting y^+ females ($\chi^2 = 10.85$; $p < 0.001$). This last result may however be explained as resulting from selection on the F_1 females to

Table 6. Comparison of inbred and outbred stocks by female-choice tests. The number of homo- and heterogamic copulations are presented. Divergence from random mating was tested by chi-square.

Female	Males	No. of trials	Homogamic	Heterogamic	p
y^+	y^+ ; y^1	10	76	4	<0.001
y^1	y^1 ; y^+	10	44	36	N.S.
y^+	y^+ ; y^2	10	51	29	<0.05
y^2	y^2 ; y^+	10	33	47	N.S.
y^{2o}	y^{2o}; y^+	10	27	53	<0.01
y^+	y^+ ; y^{2o}	10	47	33	N.S.
y^{1o}	y^{1o}; y^+	10	23	57	<0.001
y	y^+ ; y^{1o}	10	78	2	<0.001
y^{+o}	y^{+o}; y^{1o}	10	72	8	<0.001
y^1	y^1 ; y^{+o}	5	20	20	N.S.
y^{+o}	y^{+o}; y^1	10	64	16	<0.001

accept the mutant males' courtship since the wild-type se chromosome is passed on during the creation of the y^{+o} stock, but not the y^{1o} stock. Overall, then, these results agree with those of Dow (1976b) and Oakeshott and Hayman (1979). That is, in these outbreeding experiments it is not possible to separate genetic determinants of the y^1 females' receptivity to y^1 male courtship from the y locus, which may indicate that the females' receptivity is also part of the y^1 phenotype.

Table 7. Comparison of inbred and outbred stocks by male-choice tests. The number of homo- and heterogamic copulations are presented. Divergence from random mating was tested by chi-square.

Male	Females	No. of trials	Homogamic	Heterogamic	p
y^+	y^+ ; y^1	10	50	30	< 0.05
y^+	y^+ ; y^2	10	51	29	< 0.05
y^1	y^1 ; y^+	10	70	10	< 0.0
y^2	y^2 ; y^+	10	34	46	N.S.
y^{2o}	y^{2o} ; y^+	10	36	44	N.S.
y^+	y^+ ; y^{2o}	10	42	38	N.S.
y^{1o}	y^{1o} ; y^+	10	73	7	< 0.001
y^+	y^+ ; y^{1o}	10	48	32	N.S.
y^{+o}	y^{+o} ; y^1	5	27	13	< 0.05
y^1	y^1 ; y^{+o}	10	49	31	< 0.05

3.3b Effects of the Various Alleles

The effects on mating behaviour of a series of type-2 alleles and the type-1 allele $\underline{y}^{18cH1}$ were examined. Table 8 shows the pigmentary effects of these alleles on various structures (see also Nash and Yarkin 1974). Tables 9 and 10 present the results of single-pair and female-choice tests examining the ability of mutant males to mate with wild-type females. The $\underline{y}^{18cH1}$ males were as disadvantaged as $\underline{y}^1$ males. Thus, together with $\underline{y}^{59B}$ (Burnet and Wilson 1980), all three type-1 mutants examined have been similarly extremely disadvantaged. Among the type-2 mutants, $\underline{y}^{3d}$ and perhaps

Table 8. Pigmentary effects of the yellow alleles on various structures. Extent of pigmentation is scaled from 0 (yellow) to 5 (wild-type). y^{18cH1} is a type-1 allele so, as with y^{1}, is completely yellow. The rest are type-2 alleles which show pattern mosaicism.

	Proboscis			Body			Male
Allele	bristles	Aristae	Antennae	bristles	Wings	Sexcombs	Genitalia
y^{18cH1}	0	0	0	0	0	0	0
y^{3d}	1	1	0	0	1	5	0
y^{34c}	2	4	0	5	3	5	2
y^{td}	3	4	1	5	4	5	2
y^{2S}	1	5	1	5	1	5	2
y^{v2}	3	5	1	5	4	5	4
y^{bg}	2	5	0	5	4	5	3
y^{2}	4	5	5	5	1	5	2

y^{34c} were partially disadvantaged. These are also the two alleles in which the yellow phenotype is most strongly expressed in most structures (Table 8). Although the remaining type-2 mutants showed some disadvantage, this may not be part of the mutant phenotype since the outbreeding regimes may not have been fully successful. This possibility is illustrated by the finding that outbred y^{2} males here had no disadvantage (perhaps even an advantage), but earlier (3.3a) were slightly disadvantaged, despite having been outbred longer in the earlier experiments. This may also explain Burnet and Wilson's (1980) finding that

Table 9. Single-pair tests of various mutant males with wild-type females. Thirty trials were conducted for each genotype. N_5 is the no. copulating in 5 min and N_{30} the no. in 30 min. The mean and standard deviation is calculated for the courtship duration transformed to logarithms for pairs copulating within 30 min. The y^{18cH1} males mated too seldom to be included.

Male genotype	N_5	N_{30}	Mean ± S.D. (log s)
y^{18cH1}	1	3	-
y^{3d}	1	7	2.7022 ± 0.1957
y^{34c}	14	25	2.4337 ± 0.3401
y^{td}	14	27	2.4322 ± 0.3787
y^{2S}	17	28	2.3363 ± 0.3947
y^{v2}	19	29	2.2476 ± 0.4465
y^{bg}	23	26	2.2009 ± 0.2764
y^{2}	27	29	2.1007 ± 0.2889
y^{+}	23	29	2.2886 ± 0.3153

y^2 males are disadvantaged.

Because y^{3d} males had wild-type sexcombs, yet were disadvantaged, yellow expression in the sexcombs is probably not responsible for the disadvantage of type-I mutants. Similarly, y^2 males, despite yellow proboscides, were not disadvantaged. These results support Burnet and Wilson's (1980) conclusion that the pigmentary or cuticular effects of the yellow mutant are not responsible for their mating disadvantage.

Table 10. Comparison of various mutant males with wild-type males in female-choice tests. The number of homo- and heterogamic copulations are presented. Wild-type females were used throughout. Divergence from random mating was tested by chi-square.

Male genotype compared	No. of trials	Homogamic	Heterogamic	p
y^{18cH1}	10	79	1	< 0.001
y^{3d}	10	58	22	< 0.001
y^{34c}	10	51	29	< 0.05
y^{td}	10	49	31	< 0.05
y^{2S}	10	43	37	N.S.
y^{v2}	10	49	31	< 0.05
y^{bg}	10	45	35	N.S.
y^{2}	10	34	46	N.S.

3.3c Importance of Various Stimuli

Previous authors have often concentrated on the wing vibration component of the males' courtship. Wilson et al. (1976) found that the pulse song component of this wing vibration had not changed in their undesignated mutant males. This is confirmed here for $\underline{y}^{1}$ males whose interpulse interval was 33.2 $\pm$ 2.8 s (N = 10) compared with 32.2 $\pm$ 2.6 s (N = 18) for $\underline{y}^{+}$ males. The sine song component was also examined and again no significant change was found. The sound frequency was 158.7 $\pm$ 12.7 Hz (N = 9) for the $\underline{y}^{1}$ males and 151.7 $\pm$ 8.5 Hz (N = 13) for $\underline{y}^{+}$ males ($t = 1.47$: $p > 0.05$).

Table 11. Effect of removal of various stimuli in no-choice mass-mating tests. The no. of females dissected (N) and the percentage inseminated (%) in 1, 2 and 48 h tests are presented. Symbols : w - wingless, a - aristaeless, A - antennaeless.

		1 hour		2 hour		48 hour	
Female	Male	N	%	N	%	N	%
y^+	y^+	128	90.0	100	98.0	152	97.0
y^+	y^+w	150	61.4	96	93.7	-	-
y^+a	y^+	99	77.0	-	-	-	-
y^+A	y^+	-	-	184	15.8	53	96.0
y^1	y^1	97	81.5	97	90.0	-	-
y^1	y^1w	147	55.0	149	72.0	-	-
y^1a	y^1	-	-	50	80.0	-	-
y^1A	y^1	-	-	50	22.0	48	81.0
y^1	y^+	97	90.7	45	98.0	-	-
y^1	y^+w	100	35.0	98	72.5	-	-
y^+	y^1	148	27.7	98	46.0	109	85.0
y^+	y^1w	42	7.2	95	8.4	-	-
y^+a	y^1	-	-	100	31.0	-	-
y^+A	y^1	-	-	48	0.0	49	37.8
y^{1o}	y^{1o}	52	86.5	30	97.0	-	-
y^{1o}	$y^{1o}w$	50	30.0	96	49.0	-	-
y^+	y^{1o}	-	-	98	15.3	-	-
y^+	$y^{1o}w$	-	-	102	3.9	-	-

A more general and conclusive approach to the problem of which aspects of the mutant males' courtships have changed is to examine whether removal of the structures involved in either transmission or reception of courtship stimuli removes the mutants' disadvantage relative to wild-type. The results of a series of such experiments are presented in Table 11. In agreement with Mayr (1950) and Bastock (1956), $\underline{y}^1$ males were still at a disadvantage with antennaeless females. This also applied with aristaeless females. When the males' wings were removed the $\underline{y}^1$ males were still at a disadvantage. Clearly, the $\underline{y}^1$ males' courtship deficiency or change does not involve any auditory stimuli or any other stimuli transmitted via the males' wings or received via the females' aristae or antennae.

3.3d The Mutant Females' Receptivity

The results presented in Table 11 also show that the $\underline{y}^1$ females still require the stimuli normally transmitted by the males' wings or received by their aristae or antennae since their mating propensity was reduced in a similar fashion to that of $\underline{y}^+$ females when these were removed. These results also suggest that the $\underline{y}^1$ females are not just generally more receptive. That they are not generally less discriminating is demonstrated by the results of a series of tests with males of the two closely related species, D. simulans and D. mauritiana (Table 12). The $\underline{y}^1$ females were not significantly more disposed to interspecific mating. Similarly, Pinsker and Doschek (1980) found that yellow D. subobscura females, the $\underline{y}$ males of which are disadvantaged in a similar fashion to D. melanogaster (Rendel 1945), are not more disposed to mating with D. pseudoobscura. The change in the mutant females

Table 12. Receptivity of $\underline{y}^1$ and $\underline{y}^+$ females to non-conspecific courtship. The number of females dissected (N) and the percentage inseminated (%) in 48 h no-choice mass-mating tests are presented.

Female	Male	N	%
y^+	D. simulans	202	15.4
y^+	D. simulans (wingless)	92	1.1
y^+	D. mauritiana	208	3.4
y^1	D. simulans	240	21.7
y^1	D. simulans (wingless)	98	0.0
y^1	D. mauritiana	107	2.8
y^{1o}	D. simulans	102	17.6
y^{1o}	D. mauritiana	57	1.4

appears to correspond specifically to whatever has changed in the mutant males' courtship.

3.3e What Has Changed in the Males?

Burnet and Wilson (1980) concluded by noting that both their disadvantaged males ($\underline{y}^{59B}$ and $\underline{y}^{b1}$) appeared to have difficulty completing attempts at copulation (see Fig. 9). This was continuously observed in the $\underline{y}^1$ males in this study and is quantified for the various alleles in Table 13. The $\underline{y}^1$ and $\underline{y}^{18cH1}$ males seldom made genitalic contact with the wild-type females. Although it is not very clear in these results, $\underline{y}^{3d}$ males also had some difficulty. The $\underline{y}^+$ and $\underline{y}^2$ males on the other hand regularly completed copulation attempts (see Fig. 9). The rest of the alleles were intermediate. This trend remains when the

Table 13. Frequency of complete copulation attempts by various mutant males courting wild-type females. N is the no. of courtships observed. Each was observed until copulation or for four min. Frequency is expressed as no. of attempts per min. Frequency 1 is the total and frequency 2 is excluding those attempts which led to copulation.

Allele	N	Total min of courtship	Frequency 1	Frequency 2
y^{18cH1}	10	40.0	0.05	0.05
y^{1}	10	39.4	0.05	0.03
y^{3d}	10	31.2	0.29	0.16
y^{34c}	10	15.1	0.73	0.20
y^{td}	5	17.2	0.52	0.47
y^{2S}	10	21.9	0.55	0.32
y^{v2}	5	11.8	0.51	0.25
y^{bg}	5	13.4	0.45	0.23
y^{2}	10	21.3	1.08	0.70
y^{+}	10	21.3	1.36	0.99

attempts which were successful in leading to copulation are excluded (Table 13).

This phenomenon is explored further in Table 14. The results for $\underline{y}^{*}$, $\underline{y}^{1}$ and $\underline{y}^{2}$ males courting $\underline{y}^{+}$ females are similar to those above except that the $\underline{y}^{+}$ males were even more competent. The $\underline{y}^{1}$ males were more competent with $\underline{y}^{1}$ females, even when the successful copulations are excluded, again suggesting that $\underline{y}^{1}$ females

are somenow adapted to the y^1 males. That this y^1 characteristic is shared by y^{1o} but not y^{+o} males suggests that it is part of the y^1 phenotype.

Table 14. Frequency of complete copulation attempts in various pairings. The tests with young females (Y) (6 - 9 h old) and foretarsiless (F) and/or sexcombless (S) males were 5 min long. The rest were 10 min long. Other details are given in Table 13.

Female	Male	N	Total min of courtship	Frequency 1	Frequency 2
y^+	y^+	25	50.0	3.72	3.26
y^+	y^1	25	228.4	0.08	0.06
y^+	y^2	25	43.7	1.42	0.88
y^1	y^1	25	66.4	0.66	0.33
y^2	y^2	25	60.4	0.81	0.41
y^+	y^{+o}	9	11.4	2.11	1.32
y^{+o}	y^{1o}	20	184.8	0.10	0.08
y^+Y	y^+	10	50.0	0.54	0.54
y^+Y	y^1	10	50.0	0.00	0.00
y^+	y^+F	10	21.2	1.23	0.75
y^+	y^+FS	5	25.0	0.00	0.00
y^+	y^+S	6	30.0	0.00	0.00

There seem to be at least four possible explanations of this phenomenon. which are not mutually exclusive. 1) As Burnet and Wilson (1980) suggest, the $\underline{y}^1$ males may have some internal mechanical (neural or muscular) problem which prevents most attempts at copulation. 2) The $\underline{y}^1$ males may react incorrectly to some characteristic of the $\underline{y}^+$ females, perceived perhaps via the foretarsi during rubbing of the female's abdomen (Spieth 1966; Cook 1975) or via the proboscis during licking, and so not complete attempts at copulation. 3) Despite the demonstration by Bastock (1956) and Wilson et al. (1976) that $\underline{y}^+$ females direct as many rejection responses at $\underline{y}^1$ males as at $\underline{y}^+$ males, $\underline{y}^+$ females may be responding differently to $\underline{y}^1$ males and so avoid or prevent their copulation attempts more effectively. 4) The $\underline{y}^1$ males may simply be less competent in overcoming the $\underline{y}^+$ females' rejection responses.

In an attempt to examine some of these possibilities $\underline{y}^1$ and $\underline{y}^+$ males were observed courting young $\underline{y}^+$ females (6 - 9 h old) and decapitated $\underline{y}^+$ females (see Spieth (1966) and Cook (1975) for details of the method), both of which should reject the $\underline{y}^1$ and $\underline{y}^+$ males equally. As Table 14 shows, even $\underline{y}^+$ males had some difficulty in making genitalic contact with the highly unreceptive young females, but $\underline{y}^1$ males were completely incompetent. The results with decapitated females were similar, and when the males courted the anterior end of the decapitated female, $\underline{y}^1$ males were still unable to complete attempts at copulation. This last observation suggests that explanations 1 and/or 2 must be correct to some extent, but the other two also support 4.

Explanation 3 is supported by frequent observations of a rejection response used by $\underline{y}^+$ females only in response to



Figure 9. Licking and attempted copulation in Drosophila melanogaster. The male licks the female's genitalia while grasping her abdomen with the sexcombs on his foretarsi (above). At the same time he curls his abdomen underneath his body. He then releases his proboscis and sexcombs and swings his abdomen through to make genitalic contact (below).

courtship by $\underline{y}^{1}$ and $\underline{y}^{18cH1}$ males. In a behaviour called "curling" (Connolly and Cook 1973) the female lifts the tip of her abdomen as the male licks her genitalia. This appeared to

prevent attempts at copulation by the mutant males. The opposite "curling" action, i.e. pressing the tip of the abdomen against the substrate, is used by unreceptive $\underline{y}^+$ females in prolonged courtships by $\underline{y}^+$ males, as described earlier (2.3c). The $\underline{y}^1$ females did not respond to $\underline{y}^1$ males by curling, thus facilitating copulation attempts. This curling behaviour may be linked to the behaviour of the $\underline{y}^1$ males which, instead of licking briefly and then swiftly swinging through their genitalia, releasing the proboscis and making genitalic contact as $\underline{y}^+$ males do (Fig. 9), often licked for a few seconds with their genitalia curled beneath their bodies but could or would not release their proboscides and make genitalic contact. Finally, observations of $\underline{y}^+$ males minus sexcombs yielded one more point. As Cook (1977) has demonstrated, the sexcombs are used to grasp the females' abdomen during copulation attempts. Without them the males usually cannot complete copulation attempts (see also Table 14), although some eventually are able to copulate (see Table 15). When these sexcombless $\underline{y}^+$ males courted $\underline{y}^+$ females the females responded by curling their abdomens upward exactly as if courted by $\underline{y}^1$ males.

These observations suggest the involvement of the $\underline{y}^1$ males foretarsi, sexcombs or proboscides in the cause of their disadvantage, so these three structures were removed to see if this removed the disadvantage relative to $\underline{y}^+$ males. Removing the $\underline{y}^1$ males' foretarsi had no effect (Table 15A); they were just as disadvantaged. Removal of the foretarsi and the sexcombs however had a surprising effect (Table 15A). The $\underline{y}^1$ males were now equivalent to $\underline{y}^+$ males in their ability to inseminate $\underline{y}^+$ females, suggesting that the sexcombs are normally the cause of their disadvantage. The sexcombless $\underline{y}^1$ males were also

Table 15. Effect of removal of foretarsi and/or excombs on the y^1 males' disadvantage assessed by mass-mating tests. Section A is from 2 h tests, and B is from 24 h tests. The y^{+o} stock was outbred for 34 generations, and the y^{1o} for 12 generations. The number of females dissected (N) and the percentage inseminated (%) are presented. Symbols : F - foretarsiless; S - sexcombless.

Female	Male	N	%	Female	Male	N	%
	A			y^{1o}	y^{1o}	100	95.0
y^+	y^+	50	98.0	y^{1o}	$y^{1o}{}_{FS}$	300	9.3
y^+	$y^+{}_F$	50	92.0	y^+	y^{1o}	150	20.7
y^+	$y^+{}_{FS}$	200	8.0	y^+	$y^{1o}{}_{FS}$	150	8.7
y^1	y^1	100	92.0	y^1	$y^{1o}{}_{FS}$	100	5.0
y^1	$y^1{}_F$	100	92.0	y^{1o}	$y^1{}_{FS}$	200	19.5
y^1	$y^1{}_{FS}$	200	29.5	y^{1o}	$y^+{}_{FS}$	100	6.0
y^+	y^1	250	10.0				
y^+	$y^1{}_F$	150	9.3		B		
y^+	$y^1{}_{FS}$	150	10.0	y^+	$y^+{}_{FS}$	100	24.0
y^1	y^+	100	96.0	y^+	$y^1{}_{FS}$	100	33.0
y^1	$y^+{}_F$	100	92.0	y^1	$y^1{}_{FS}$	100	65.0
y^1	$y^+{}_{FS}$	150	8.0	y^1	$y^{+o}{}_{FS}$	100	64.0
y^1	$y^{+o}{}_{FS}$	200	21.5	y^1	$y^1{}_{FS}$	100	15.0

surprisingly competent with their own females, and this appeared to be due to the y^1 males since sexcombless y^+ males were as incompetent with y^1 females as with y^+ females. Sexcombless y^{+o} (34 generations) males were however similarly successful

with y^1 females suggesting that this characteristic of the y^1 males is not part of the y^1 phenotype. The results with the new y^{1o} stock (12 generations), although not as clear, also support this interpretation. This experiment was repeated with a slightly modified regime and yielded even more clearcut results (Table 15B). Somehow the y^1 males have changed to become remarkably competent without their sexcombs, but the sexcombs themselves do not appear to be the cause of their disadvantage. Tests with a more thoroughly

Table 15. Effect of removal of proboscides on the y^1 males' disadvantage assessed by mass-mating tests. Details are given in Table 15. Symbol : P - probosciless.

Female	Male	N	%	Female	Male	N	%
	A				B		
y^+	y^1	100	44	y^+	y^1	100	65
y^1	y^1	100	69	y^1	y^1	50	96
y^+	y^+P	100	50	y^+	y^+P	100	82
y^1	y^1P	100	4	y^1	y^1P	100	45
y^+	y^1P	100	0	y^+	y^1P	100	6

outbred y^{1o} stock should make this conclusive.

Finally, removal of the males' proboscides, if anything, increased the y^1 males' disadvantage (Table 16). The most surprising aspect here was the relative ease with which y^+ males without proboscides copulated. This implies that no crucial stimuli are exchanged in either direction during the licking

behaviour, and that this behaviour is not mechanically essential for the male to make copulation attempts.

3.4 Discussion

The finding that different yellow alleles cause different levels of mating disadvantage in the mutant males as reported here and by Burnet and Wilson (1980) requires a reassessment of the possible characteristics of the cause of this disadvantage. Nash (1976) proposed that the type-1 alleles affect the structural gene and hence the entire fly equally, whereas type-2 alleles, exhibiting pattern mosaicism, affect a regulatory area mapping to the right of the type-1 allele structural gene and so affect the place and time, and hence extent, of the yellow expression.

There are two ways these characteristics could explain the mutant males' disadvantage. Firstly, type-1 alleles might affect many aspects of the males' behaviour and courtship such as his locomotory activity, latency to courtship, intensity and various aspects of the courtship such as the wing vibration, licking and the attempts at copulation. Together these would greatly impair but not completely block the males' mating ability. Type-2 alleles would affect none, one or some of these aspects by affecting only particular neuronal, sensory or structural features and so exhibit a range of mating competence. Alternatively, the change in type-1 alleles might be of a specific nature, e.g. only affecting attempted copulation, and only type-2 mutants also affecting whatever neuronal or structural feature is involved would affect mating behaviour, the extent of the effect depending on the level of yellow expression.

Burnet and Wilson (1980) favoured the first explanation. Thus

they ascribed the disadvantage they found in $\underline{y}^2$ males to the increased latency to courtship they exhibited. Such an increased latency was noticed in the inbred $\underline{y}^2$ stock used here which was also partially disadvantaged. The two outbred stocks however, did not exhibit this, and one had lost all its disadvantage. This variable and uncertain nature of the outbreeding process may explain their results. They similarly ascribed the extreme disadvantage of their $\underline{y}^{bl}$ males ($\underline{y}^{bl}$ is a type-2 allele but has as extreme an effect on the mating competence of the males as do type-1 alleles) in part to the reduced intensity of the males' courtship. As noted in the Introduction this seems unlikely to be important however since earlier Wilson et al. (1976) found that males of an undesignated $\underline{y}$ stock had less intense courtship on one genetic background but not another, yet were still disadvantaged on both backgrounds. Similarly Dow (1976c) found that increased courtship intensity did not explain the improved mating competence of his selected $\underline{y}^1$ lines.

They also suggest that since the $\underline{y}^{bl}$ males, like $\underline{y}^{59B}$ males (a type-1 allele), have difficulty completing copulation attempts, this may be a major cause of their disadvantage. Present observations on $\underline{y}^1$ and $\underline{y}^{18cH1}$ confirm this as the immediate cause of the males' disadvantage. Since this is common to all the disadvantaged stocks studied so far and partly to the partially affected $\underline{y}^{3d}$ stock studied here, I propose that the second explanation postulated above is correct, i.e. there is a single common ultimate cause of these males' incomplete copulation attempts. This hypothesis would be disproved if a type-2 allele with males capable of regularly completing copulation attempts,

but nevertheless disadvantaged, was found.

This ultimate cause remains unknown, but the present results together with those of other workers allow the elimination of some possibilities. It does not involve stimuli transmitted by the males' wings, foretarsi, proboscides or sexcombs, or received via the females' aristae or antennae, or via the males' foretarsi or proboscides. It also does not seem to involve mechanical problems during copulation attempts involving the proboscides or sexcombs. However, the question of the involvement of the sexcombs has not yet been conclusively settled. Burnet and Wilson (1980) found that their removal did not decrease the disadvantage of $\underline{y}^{01}$ males. In this study their removal removed the disadvantage of inbred $\underline{y}^{1}$ males, however this appeared not to be a part of the $\underline{y}^{1}$ phenotype. Examination of this aspect in other thoroughly outbred or newly isolated type-1 alleles would be worthwhile. The inbred $\underline{y}^{1}$ males' improved ability to copulate without sexcombs may be the result of selection to cope with another deficiency in their copulation attempts.

Burnet and Wilson's (1980) suggestion that the behavioural focus in the thoracic ganglion which controls copulation attempts (see Hall 1979) may be affected by lower levels of neural transmitters caused by the mutation (see also Burnet and Connolly 1974) remains tenable. However, since mutant females somehow accommodate to the change in the mutant, it is probably not a purely internal mechanical change. This idea could be tested using the elegant method of genetic mosaics, since most workers use yellow expression to mark the cuticle of the mosaics (e.g. Hotta and Benzer 1976; Hall 1977). Usually the tester females used are homozygous $\underline{y}$

(because attempted copulation and copulation could not be examined with wild-type tester females?), but Hall (1979) used both $\underline{y}$ and $\underline{y}^+$ females. Unfortunately, his results, as presented, are not detailed enough to examine whether mosaics with *yellow* expression in the foci in the thoracic ganglia which control attempted copulation are able to complete attempts at copulation with $\underline{y}$ but not $\underline{y}^+$ females. Indeed, the structure which is affected by *yellow* expression in males could be identified by screening for mosaics which are able to mate easily with $\underline{y}$ females, but not $\underline{y}^+$ females.

Bastock's (1956) suggestion that the $\underline{y}$ females' receptivity to $\underline{y}$ males is not part of the $\underline{y}$ phenotype but was selected for during man, generations of exclusive exposure to $\underline{y}$ males is not supported here or by Dow (1976b) and Oakeshott and Hayman (1979). Nevertheless, as Oakeshott and Hayman (1979) caution, these results do not demonstrate that the $\underline{y}$ females' receptivity is part of the $\underline{y}$ phenotype, because linkage disequilibrium may cause a considerable portion of the X chromosome to remain linked to the $\underline{y}$ locus in these outbred strains. This portion may bear the genetic determinants of the $\underline{y}$ females' receptivity, especially since Dow (1977) found that the receptivity of the $\underline{y}^+$ females successfully selected to mate with $\underline{y}$ males was partly determined at sex-linked loci. This question will only finally be settled by examination of a newly isolated type-1 allele. What has changed in the $\underline{y}$ females is also unknown, but present results suggest that they are not generally more receptive or less discriminating. If, as suggested here, the change in the mutant males is a specific one, hen the females are probably specifically correspondingly altered to accommodate this. The use of genetic mosaics may also help elucidate this female change.

Chapter 4. MATING BEHAVIOUR AND THE EVOLUTION OF DROSOPHILA MAURITIANA

4.1 Introduction

As discussed in Chapter 1, species are commonly defined in terms of their reproductive isolation from other species (Mayr 1970; Dobzhansky 1970). This isolation concept of species holds that their integrity is maintained by reproductive isolating mechanisms. As Sibley (1957), Mayr (1970) and others have noted, from this concept it is predicted that species which are spatially isolated from close relatives should have no need for isolating mechanisms, so these mechanisms should be relaxed and the behaviour normally involved in them should be variable and non-specific. Although little evidence, all involving only phenotypic variance, has been produced to support this prediction (Mayr 1970), and despite some opposing evidence (e.g. Littlejohn 1964), the isolation concept remains intact (Littlejohn 1981). Drosophila flies are particularly useful for examining this prediction because in addition to phenotypic or behavioural variation, the actual specificity of mating responses can readily be determined. This is important because phenotypic changes may not be related to the specificity of mating.

Drosophila mauritiana (Tsacas and David 1974) is a potentially interesting example of an isolated species. It is a sibling species of melanogaster and simulans from which it only differs obviously in the male genitalic morphology (Tsacas and David 1974), particularly in having narrow posterior lobes of the genital arch (see Appendix D). Other evidence confirms this close

relationship (Barnes et al. 1978; Cseko et al. 1979; Eisses et al. 1979). Two further lines of evidence indicate that mauritiana is most closely related to and probably evolved from simulans. Firstly, mauritiana and simulans chromosomes are homosequential and diffe from those of melanogaster by one major paracentric inversion and a few minor inversions (Lemeunier and Ashburner 1976). Secondly, mauritiana and simulans produce viable hybrids when crossed, the females being fertile, but they both produce only sterile males with melanogaster males and sterile females with melanogaster females (David et al.1974).

D. mauritiana is endemic to the island of Mauritius from which simulans and melanogaster are absent. They are however present on the neighbouring island of Reunion from which mauritiana is absent (David and Tsacas 1975). Mauritius and Reunion are approximately 320 km apart, and 960 km and 720 km from Malagasy, respectively. The only other melanogaster group species on Mauritius are D. ananassae and D. parabipectinata (David and Tsacas 1975). Their courtship is considerably different to that of the melanogaster subgroup (Narda 1966, 1968; Hedge and Krishnamurthy 1979).

To date there have been three studies involving mauritiana mating behaviour. 1) Schilcher and Dow (1977) apparently found that melanogaster and simulans males do not readily court mauritiana females. 2) Watanabe and Kawanishi (1979) confirmed the observation of David et al. (1974) that mauritiana males, but not females, can be crossed easily with simulans and less readily with melanogaster. 3) Cowling and Burnet (1981) reported that mauritiana males use an additional wing display (rowing),

described the courtship songs produced by their wing vibration, and examined the courtship songs of hybrids with melanogaster and simulans.

Independent data bearing on these three studies are presented here, and the interspecific mating behaviour of mauritiana with melanogaster and especially simulans is examined. Possible explanations of the evolution of mauritiana are then considered, and the implications for species concepts and speciation theory are discussed.

4.2 Materials and Methods

4.2a Flies

The Canton-S strain of melanogaster was again used. The simulans strain was collected locally (Drakensberg, 1958). Two mauritiana strains were used. The first, (1), was provided by Dr. L. Tsacas from the original collected strains. The second, (2), used after the first was lost, was an isofemale line collected on Mauritius by M. Picker in 1979, as was a Zaprionus mascariensis isofemale line used here.

The stocks were maintained as before (2.2a). Despite the constant light regime, to be consistently successful, observations and 1 h tests with mauritiana had to be conducted in the morning. Virgin flies were collected at 12 h intervals, sexed under ether, stored in 10 x 2.5 cm vials with yeasted food, and used after 3 - 4 days (Drosophila) or 5 - 7 days (Z. mascariensis). For the initial observations and the 48 h mating experiment the flies were kept isosexually 20 per vial, but otherwise 10 - 12 per vial. For the sound recordings males were kept singly for 20 - 24 h before use.

4.2b Observations

Detailed observations of individual courtships were made at 6X magnification with single pairs in the small perspex chambers described earlier (2.2c). Observations and recordings of the males' courtship songs were made on the sound recording apparatus described earlier (2.2b). All song phrases in complete courtships, i.e. those ending in copulation, were analysed to avoid sampling bias. Pulse song was analysed on a sonograph, with phrases defined as consisting of at least 4 pulses and separated from another phrase by at least 200 msec. For simulans and mauritiana all inter-pulse intervals (ipi's) were measured, and means of phrase means and means of males' means computed. For melanogaster. phrase means were measured and the mean of these used. Sine song was analysed on an oscilloscope and the sound frequency for individual phrases measured. Means were compared using two-tailed t-tests.

4.2c Experiments

All experiments were of the mass-mating, no-choice type and were conducted in food vials. There were two kinds. In the first, 10 females and 5 (Drosophila 48 h and Zaprionus 1 h tests) or 6 (Drosophila 1 h tests) males were placed in a vial for 1 or 48 h, after which the females were dissected and examined for insemination. In the second, 10 females and 10 males were observed in a vial for 10 min with appropriate observations, such as time to copulations, recorded. Results were compared using chi-square tests.

4.2d Manipulations

As before (3.2b) various structures were amputated from the flies under ether anaesthesia 20 - 24 h before use. Aristae,

foretarsi distal to or including the sexcombs, and the posterior lobes of the genital arches of the male genitalia were snipped off with the fine scissors; antennae were plucked off with watchmaker's forceps, removing at least the funiculus; and wings were cut off near the base with a scalpel. Females were decapitated with scissors and used shortly thereafter in moist vials (see Spieth 1966). Young females were collected at 3 h intervals and used after a further 6 h. Mating tests in the dark were done by swiftly placing the vials in a light-proof tin on the laboratory desk. Observations under red light (effective darkness for the flies) were conducted in a photographic darkroom.

4.3 Results

4.3a Wing Displays

The wing displays in Drosophila are described by Spieth (1974).

Table 17. Frequency of various wing displays in simulans and mauritiana (1) courtships measured as bouts per minute of courtship.

Pairing			Total	Wing display		
Female	Male	N	min	Rowing	Scissoring	Vibration
mau	mau	14	7.5	2.3	1.3	5.8
sim	sim	19	5.7	0.5	0.7	4.1
sim	mau	15	3.4	1.5	1.2	8.5
mau	sim	7	35.0	1.8	3.3	4.7

These were initially examined in single-pair observations, which lasted 5 min or until copulation. In basic organization,

foretarsi distal to or including the sexcombs, and the posterior lobes of the genital arches of the male genitalia were snipped off with the fine scissors; antennae were plucked off with watchmaker's forceps, removing at least the funiculus; and wings were cut off near the base with a scalpel. Females were decapitated with scissors and used shortly thereafter in moist vials (see Spieth 1966). Young females were collected at 3 h intervals and used after a further 6 h. Mating tests in the dark were done by swiftly placing the vials in a light-proof tin on the laboratory desk. Observations under red light (effective darkness for the flies) were conducted in a photographic darkroom.

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These were initially examined in single-pair observations, which lasted 5 min or until copulation. In basic organization,

mauritiana courtship was generally similar to that of melanogaster (see Burnet and Connolly 1974) and simulans (Manning 1959a). In contrast to simulans males however, mauritiana males exhibited relatively more rowing than scissoring (Table 17). Rowing involves extending, arcing and replacing one wing (Cowling and Burnet 1981), while scissoring is a rapid horizontal opening and closing of both wings. In the experiments reported in Chapter 2, rowing was never, and scissoring seldom observed in melanogaster. Like melanogaster, both simulans and mauritiana regularly used wing vibration, which, unlike rowing and scissoring, was present in every courtship.

This was more closely examined in single-pair observations made on the sound recording apparatus. Pairs were watched until all components had been observed or heard, or they had copulated. As before, wing vibration could be split into its components, sine and pulse song, on sound characteristics (Schilcher 1976a), and also by the manner of vibration. Sine song is generated by low amplitude vibration with the wing held horizontal while pulse song involves high amplitude vibration with the posterior margin of the wing lowered. All 34 mauritiana courtships observed contained pulse song. Two (6%) did not contain sine song, but all the remaining 32 at least started with a burst of sine song and often contained sine song thereafter. Twenty-one included rowing and 12 scissoring. Of 20 simulans courtships, again all contained pulse song, 2 (10%) contained no sine song and 13 started with sine song, 6 included rowing, and 9 scissoring. Again mauritiana males exhibited relatively more rowing than scissoring. These two behaviours were however only present in up

to half the courtships, and all those without them led to copulation. In the repeat mating experiment in Chapter 2, 35 (7%) of 492 melanogaster courtships contained no sine song. The sequence of song bursts was recorded in 202 of these courtships and only 2 started with sine song. Thus although sine song is not always present and so is not essential for copulation, mauritiana and simulans appear to have changed from melanogaster in that most of their courtships started with sine song.

4.3t Courtship Songs

The analysis of the two types of courtship song produced by the two modes of male wing vibration is presented in Table 18. The results for melanogaster and simulans are within the limits of variability expected from the literature (e.g. Ewing and Bennet-Clark 1968; Schilcher and Manning 1975; Schilcher 1976a; Cowling 1980; Kawanishi and Watanabe 1980; Cowling and Burnet 1981), except that the sine song frequency of simulans was considerably lower than that reported by Cowling and Burnet (1981). As they reported, melanogaster pulse song interpulse interval (ipi) is very constant, simulans is somewhat variable, but mauritiana ipi is very variable (see also Fig. 10). These mauritiana pulse song phrases were separable into four types. Type 1 (15 of 62) started with irregularly and widely spaced pulses which became closer and more regular until the ipi was approximately 30 msec, i.e. similar to melanogaster ipi (e.g. Fig. 10C). Type 2 (7) phrases were similar to the first irregular part of Fig. 10C, type 3 (12) was similar to the second part with regularly decreasing ipi's, and type 4 (28) had constant, short ipi's (e.g. Fig.10D). The mean ipi and level of variability are both

Table 18. Analysis of the pulse song inter-pulse interval (ipi) and sine song frequency of melanogaster, simulans and mauritiana courtship songs.

Song type and parameter	Species	Type of analysis	N	Mean ± S.D.
	mel	phrase means	17	34.6 ± 2.2
	sim	all intervals	295	50.2 ± 12.8
Pulse song	sim	phrase means	19	50.1 ± 6.4
ipi (msec)	mau	all intervals	822	51.6 ± 29.3
	mau	phrase means	62	52.3 ± 21.9
	mau	male means	10	54.8 ± 12.6
Sine song	mel	phrase means	13	151.6 ± 8.5
frequency	sim	phrase means	12	176.0 ± 6.6
(Hz)	mau	phrase means	24	198.9 ± 12.7

higher than Cowling and Burnet (1981) reported, probably because they used a lower cut-off point to define a phrase (150 msec versus 200 msec). In fact the variability is even greater if this restriction is not applied rigidly. For example, the first part of Fig. 10C was not actually included in the analysis in Table 18 because the ipi's are longer than 200 msec. As Cowling and Burnet (1981) discuss, it is difficult to compare statistically these mauritiana parameters with those for melanogaster and simulans, but it is clear that mauritiana pulse song is extremely variable.

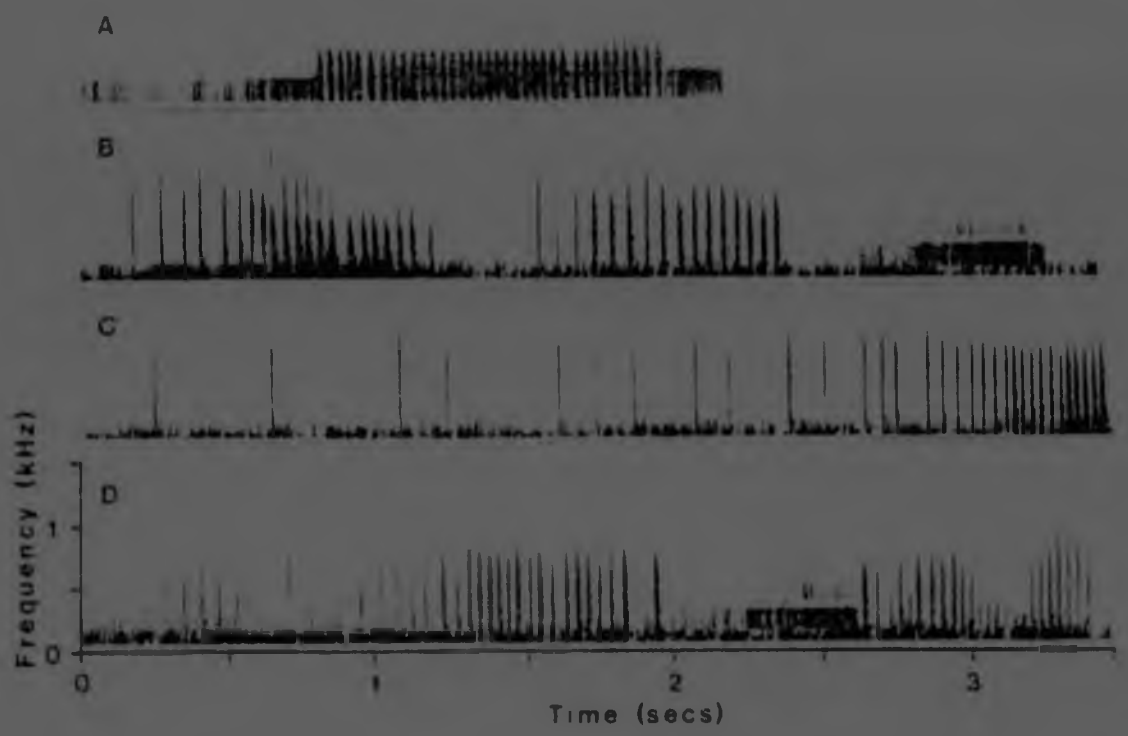

Figure 10. Sonagraphs of Drosophila courtship songs. A - D. melanogaster pulse song. B - Two phrases of D. simulans pulse song with sine song at the end. C - A variable D. mauritiana pulse song phrase. D - Two regular D. mauritiana pulse song phrases separated by sine song.

The mauritiana sine song frequency found here is similar to th ecorded by Cowling and Burnet (1981) and, because the simulans sine song frequency found here is lower than theirs, the mauritiana sine song frequency is significantly higher than that of simulans ($t = 5.15$; $p < 0.001$). It is also more variable ($F = 4.99$; $p < 0.01$). The wingbeat frequency of free-flying mauritiana males measured here as 287.4 $\pm$ 10.3 Hz ($N = 15$) is also higher than that reported for simulans (252.7 Hz) and melanogaster (218.3 Hz) (Schilcher and Manning 1975).

4.3c Light Dependency

Some species of Drosophila will not mate in the dark and others, e.g. simulans, are partially inhibited by lack of light

Table 19. Number of females dissected (N) and percentage inseminated (%) in light, dark, and with wingless or control (etherized only) males in intraspecific pairings of melanogaster, simulans, mauritiana and Z. mascariensis in 1 h tests.

Species	Condition	N	%
mel	light	100	91
mel	dark	50	94
mel	ether	100	89
mel	wingless	100	72
sim	light	98	83
sim	dark	199	73
sim	ether	100	81
sim	wingless	100	66
mau	light	167	41
mau	dark	245	8
mau	ether	100	55
mau	wingless	200	25
sim	light	100	90
sim	wingless	100	80
sim	dark	100	79
sim	dark and wingless	100	36
mau	light	200	80
mau	wingless	100	41
mau	dark	420	18
mau	dark and wingless	200	6
mas	light	100	88
mas	wingless	280	72
mas	dark	300	36
mas	dark and wingless	200	29

Table 19. Number of females dissected (N) and percentage inseminated (%) in light, dark, and with wingless or control (etherized only) males in intraspecific pairings of melanogaster, simulans, mauritiana and Z. mascariensis in 1 h tests.

Species	Condition	N	%
mel	light	100	91
mel	dark	50	94
mel	ether	100	89
mel	wingless	100	72
sim	light	98	83
sim	dark	199	73
sim	ether	100	81
sim	wingless	100	66
mau	light	167	41
mau	dark	245	8
mau	ether	100	55
mau	wingless	200	25
sim	light	100	90
sim	wingless	100	80
sim	dark	100	79
sim	dark and wingless	100	36
mau	light	200	80
mau	wingless	100	41
mau	dark	420	18
mau	dark and wingless	200	6
mas	light	100	88
mas	wingless	280	72
mas	dark	300	36
mas	dark and wingless	200	29

(Spieth and Hsu 1950: Grossfield 1971). This was examined in the present species and Z. mascariensis by placing flies in a vial and later dissecting and examining the females for insemination. Table 19 presents the results of two such 1 h mass-mating experiments. The first (top) involved mauritiana (1) and also a control for this and later experiments for the effect of ether anaesthesia. The second (bottom) tested whether the males' wings are still important in the dark, and used mauritiana (2) and Z. mascariensis. The results for melanogaster and simulans are similar to those of Manning (1959a), indicating that melanogaster mating is largely light-independent and the males' wings provide non-visual stimuli, while simulans is partially light-dependent. The observation that removal of the males' wings in darkness reduces simulans mating suggests that the wings still provide some non-visual stimuli. The two strains of mauritiana differed in overall mating propensity, but were otherwise similar. Clearly mauritiana depends considerably on visual stimuli for mating, but non-visual stimuli still play a role because the wings are still important in the dark. Mating of Z. mascariensis is similarly greatly inhibited by lack of light, but here the males' wings do not appear to be important in the dark.

To examine the effect of the removal of light, flies were observed in vials under red light. While the simulans males courted readily under red light, the mauritiana (2) males hardly courted at all. Also, three spontaneously mutated white eye mauritiana males were never observed to court females and attempts to establish lines from them were unsuccessful. Other authors have made similar observations with the light-dependent species D. subobscura (e.g. Rendel 1945). Furthermore, mauritiana males,

unlike those of melanogaster and simulans, seldom courted decapitated females, and then only if they approached from behind. This is similar to the behaviour of D. subobscura and D. auraria (Spieth 1966; Grossfield 1970). Thus mauritiana males require some visual stimulus from the female for initiation of courtship, which probably explains most of the light-dependence of this species.

4.3d Male Initiation of Courtship

In Drosophila the males initiate courtship (Spieth 1952). This involves an initial visual reaction, which as shown above is more important in mauritiana, by which the male orients to the female. Volatile chemicals from the female may be important in stimulating male courtship (see Tompkins et al. 1980), but this has yet to be satisfactorily established (see Appendix A). The mauritiana males in the experiments below often appeared reticent to proceed to the next courtship step, i.e. tapping, with melanogaster females. This may indicate olfactory discrimination because the females of these species are so similar that visual discrimination seems unlikely. Manning (1959b) concluded that the male receives contact chemical stimuli by tapping the female with his foretarsi. He found that melanogaster and simulans males are more likely to court interspecifically when foretarsiless or with young females (which he suggested do not produce the chemicals). This was examined with mauritiana in single-pair 10 min observations (Table 20). All intraspecific pairings led to courtship, and most to copulation. In the interspecific pairings, in agreement with most authors (Manning 1959b; Wood and Ringo 1980) melanogaster courted simulans, but not vice-versa.

Table 20. Extent of courtship and number of copulations (N_2) in courtships of melanogaster, simulans and mauritiana (1) observed in 10 min single-pair tests. Courtship is defined as wing vibration to attempted copulation. Symbols : F - foretarsiless, Y - young.

Pairing			Extent of courtship			
Female	Male	N	Nil	Start-Stop	Full	N_2
mel	mel	25	0	0	25	23
sim	sim	10	0	0	10	10
mau	mau	24	0	0	24	24
mel	sim	10	7	3	0	0
sim	mel	12	3	1	8	0
mel	mau	15	15	0	0	0
mau	mel	20	7	12	1	0
sim	mau	18	2	0	16	15
mau	sim	10	1	0	9	0
mel	mel F	10	0	0	10	10
mau	mau F	5	1	0	4	1
mel	mau F	5	5	0	0	0
mel Y	mel	10	0	0	10	0
mau Y	mel	10	0	0	10	0
mau Y	mau	10	7	1	2	0
mel Y	mau	10	10	0	0	0

As Schilcher and Dow (1977) reported, melanogaster did not readily court mauritiana (but see Appendix E). Some males did

start courting, apparently because they did not tap properly initially, but most stopped later upon tapping the female repeatedly and vigorously. This reticence on the part of the melanogaster males was removed with young mauritiana females. Foretarsiless males were too disabled to get near the mauritiana females, which avoided the melanogaster males, so these could not be tested. Under no circumstances could mauritiana males be induced to court melanogaster females in these 10 min tests. Furthermore, in contrast to melanogaster and simulans males (Manning 1959b) mauritiana males were even reticent to court their own young females. Thus mauritiana males appear to be generally more specific in their initiation of courtship.

In contrast to Schilcher and Dow (1977) the males courted readily in both reciprocal pairings of mauritiana and simulans. While most interspecific pairings, even if leading to courtship, did not lead to copulation, mauritiana males and simulans females mated easily (Table 20). These interspecific pairings are examined below.

4.3e Interspecific Mating

Various authors (e.g. Mayr and Dobzhansky 1945; Mayr 1946, 1950; Ehrman 1959; Narda 1968) have attempted, with varying success, to determine by experimental removal of stimuli which stimuli differ between species, and so lead to lack of mating in interspecific pairings. This was attempted here in 48 h mass-mating tests. Three observations can be made from the initial intraspecific tests (Table 21). Firstly, mauritiana flies, and particularly the females, were generally more sensitive to removal of various stimuli. This is apparently not simply a greater

Table 21. Percentage insemination in 48 h mass-mating no-choice intraspecific tests. Symbols for this and subsequent tables : E - etherized, D - dark, F - forctarsiless, S - sexcombless, a - aristaeless, A - antennaeless, w - wingless.

Pairing				Pairing			
Female	Male	N	%	Female	Male	N	%
mel	mel	152	97	sim A	sim	45	82.5
mel	mel W	100	98	sim	sim F	52	94
mel a	mel	49	98	sim	sim FS	55	62
mel A	mel	53	96	mau	mau	102	95
mel	mel F	100	96	mau D	mau D	100	86
mel	mel FS	48	33.4	mau	mau W	98	94
sim	sim	100	91	mau a	mau	89	73
sim D	sim D	104	95	mau A	mau	86	72
sim	sim W	96	98	mau	mau F	33	82
sim a	sim	100	98	mau	mau FS	30	57

sensitivity to amputations because removal of the aristae or antennae had similar effects, yet the latter is a more severe amputation. Secondly, all three species were most seriously affected by removal of the sexcombs which Cook (1977) has demonstrated are necessary for the male to grasp the female during attempts at copulation. Thirdly, the inhibition of mauritiana mating in the dark disappeared in these longer tests indicating that it is facultative.

In the interspecific tests, removal of stimuli generally

Table 22. Percentage insemination in 48 h mass-mating, no-choice interspecific tests. See Table 21 for symbols.

Pairing				Pairing			
Female	Male	N	%	Female	Male	N	%
mel	sim	302	10.3	mel	mau FS	145	17.2
mel D	sim D	52	0	mau	mel	101	0
mel	sim W	92	1.1	mau D	mel D	50	0
mel a	sim	50	2	mau	mel W	49	0
mel A	sim	93	0	mau a	mel	50	0
mel	sim F	150	22	mau A	mel	44	0
mel	sim FS	150	24	mau	mel F	48	0
sim	mel	135	1.5	mau	mel FS	50	0
sim D	mel D	48	0	mau A	mel FW	44	0
sim	mel W	86	1.2	mau	sim	218	4.6
sim a	mel	48	2.1	mau D	sim D	151	6.0
sim A	mel	100	0	mau	sim W	239	0
sim	mel F	50	4	mau a	sim	49	0
sim	mel FS	47	0	mau A	sim	50	0
mel	mau	208	3.4	mau	sim F	99	1
mel	mau W	175	6.3	mau	sim FS	95	0
mel D	mau D	103	2	sim	mau	148	92.6
mel a	mau	50	6	sim	mau W	106	85
mel A	mau	48	0	sim a	mau	47	87.5
mel	mau F	146	24.7				

lowered the level of insemination (Table 22). The exception was removal of the foretarsi of simulans and mauritiana males when courting melanogaster females. These are the two pairings in which courtship was seldom observed in the 10 min tests. Presumably the fo[illegible]less males eventually did court in these 48 h tests, thus raising the level of insemination. In agreement with Watanabe and Kawanishi (1979), melanogaster females were more likely to mate interspecifically than were simulans females. while mauritiana females were never observed to mate with melanogaster males and seldom with simulans males. That removal of the males' wings did not increase the level of insemination in this last pairing suggests that the differences observed earlier in the wing displays of these two species are not crucial. Finally, as expected, pairing simulans females and mauritiana males led to interspecific levels of insemination. This phenomenon is examined below.

4.3f D. mauritiana Males and D. simulans Females

This pairing regularly led to copulation in the single-pair observations presented earlier. Indeed the courtship durations, transformed to logarithms (0.897 ± 0.591 log s ; N = 15) were even shorter than for intraspecific simulans (1.468 ± 0.341 log s; N = 10) and mauritiana (1.474 ± 0.481 log s; N = 24) courtships. It immediately became apparent however that these simulans females were not content to mate with the mauritiana males. In all 15 observations, immediately upon intromission the female began vigorous, even violent, efforts to dislodge the male. This frequently involved violent flying around the observation chamber whereafter the female attempted to prize the male off with her hindlegs, and all eventually managed to remove the male. At no

Table 23. Percentage insemination of simulans and mauritiana (2) females paired with mauritiana (2) males in 1 h tests. See Table 21 for symbols.

Pairing		Number	Percentage
Female	Male	dissected	inseminated
sim	mau E	100	42
sim	mau W	100	27
sim D	mau D	100	3
sim	mau F	100	3
sim	mau FS	100	7
mau	mau F	50	1
mau	mau FS	100	7

point did the males attain the normal copulation position between the females' wings and only held on via their genitalia. Consequently these copulations were rather short (4.81 ± 2.09 min; N = 14). Normal mauritiana copulations were considerably longer (11.44 ± 2.60 min ; N = 10; t = 6.92; p < 0.001), as are simulans copulations (16.87 min; Spieth and Hsu 1950).

This was examined further in three experiments. The first (1 h mass-mating tests) showed that removal of stimuli decreased the rate of insemination in a similar fashion to intraspecific tests (Table 23). Although most of these results can be attributed to impairment of the males' ability or propensity to court, that wingless males were less successful implies that the females usually used the stimuli from the males' wing displays.

This was examined further in the second experiment by observing 10 pairs in a vial for 10 min and recording times to copulations (which were defined as lasting at least 15 s). Apparently stimuli from the males' wings and stimuli received by the females' antennae were still used by the simulans females, yet in all three conditions the mauritiana males were still more able to copulate with the simulans females than were simulans males (Table 24). The females still objected to copulation with the mauritiana males.

Table 24. Percentage copulating (%) and time to copulations (mean ± S.D. ; s) in 10 min observations of 10 pairs of simulans and mauritiana flies. See Table 21 for symbols.

Pairing				Time to
Female	Male	N	%	copulations
sim	mau	60	90	256 ± 146
sim	mau W	50	64	316 ± 160
sim A	mau	100	25	348 ± 148
sim	sim	50	80	323 ± 98
sim	sim W	40	35	431 ± 116
sim A	sim	100	14	398 ± 142
mau	mau	40	95	287 ± 120

The final experiment involved removal of as many potentially different stimuli as possible in an attempt to remove whatever causes the females to object (Table 25). None of the treatments

Table 25. Level of courtship, copulation and female objection in pairings of *simulans* females and *mauritiana* males. See Table 21 for symbols, plus; R - red light, G - posterior lobe of genital arch removed, P - probosciless, H - decapitated, Y - young.

Pairing				
Female	Male	Courtship	Copulation	Objection
sim	mau	frequent	frequent	vigorous
sim	mau W	frequent	frequent	vigorous
sim W	mau	hesitant	seldom	vigorous
sim W	mau W	hesitant	seldom	vigorous
sim A	mau	frequent	frequent	vigorous
sim	mau F	seldom	seldom	vigorous
sim	mau FS	seldom	seldom	vigorous
sim R	mau R	seldom	seldom	vigorous
sim R	mau R	seldom	seldom	vigorous
sim	mau G	frequent	frequent	vigorous
sim	mau P	frequent	seldom	vigorous
sim H	mau	seldom	none	-
sim Y	mau	none	-	-

led to normal copulations suggesting that either a combination of these factors or something else is responsible for the females objecting. These observations confirmed the impression, gained from the earlier observations and experiments, that the *mauritiana* males, which make extremely quick attempts at copulation, were simply taking the *simulans* females by surprise. In normal intraspecific courtship in all three species, males sometimes

copulated rapidly whereupon the females would object briefly with loud buzzing from rapid vibration of both wings and attempts to prize the male off (see also Manning 1967a). The reaction of simulans females to mauritiana males may be an extreme form of this resulting from the females' perception of the absence or incorrectness of, as yet unidentified, stimuli. In this larger sample, some females eventually did stop objecting and the males achieved the normal copulation position. These were all particularly long copulations (10.9 ± 3.8 min; N = 25) compared to those in which the female managed to remove the male (2.9 ± 2.8 min; N = 74). There was no obvious reason for this female acquiescence, i.e. it did not result from particular treatments in Table 25. Perhaps it simply resulted from the males having been able to fully insert and secure their genitalia before the females started objecting. Although many of the short copulations would not lead to sperm transfer (see Hall et al. 1980), some do because in the 48 h tests high insemination rates were obtained (Table 22). This is probably also the result of repeat copulations, which were occasionally observed in this experiment.

Finally, in agreement with the earlier observations with melanogaster and mauritiana females, mauritiana males would not court young simulans females, and seldom courted decapitated females, or normal females in red light (Table 25).

4.4 Discussion

4.4a Mating Behaviour

D. mauritiana mating behaviour has changed from that of simulans in a number of aspects. For the initiation of courtship, in addition to the contact chemicals presumably gained by

tapping the female, visual, and perhaps olfactory, stimuli are more important for mauritiana males than for either simulans or melanogaster. D. mauritiana males are generally more specific in their responses. These initial recognition steps by the male usually differ between species (Spieth 1949, 1952; Manning 1959b; Schilcher and Dow 1977; Wood and Ringo 1980). The potential importance of such differences is shown by the finding that removal of the foretarsi from simulans and mauritiana males was the only manipulation which increased their rate of insemination of melanogaster females, which they would normally seldom court. As evidenced by the differential responses of melanogaster males, mauritiana females differ to some extent from simulans females of the strain used here in the stimuli eliciting courtship, but the mauritiana and simulans males do not perceive this difference. Other strains of simulans may differ more (Schilcher and Dow 1977). Nevertheless mauritiana clearly has not diverged from simulans sufficiently to exclude interspecific courtship. Other closely related (and sympatric) species pairs similarly court interspecifically (e.g. Wallace and Dobzhansky 1946; Plurka and Potter 1977).

The mauritiana males' courtship has also changed in a number of aspects. Firstly, in addition to scissoring, they use another apparently visual wing display, rowing, which simulans uses less, or not at all (Cowling and Burnet 1981). It is difficult to examine the significance of this behaviour because the males will not court readily in the dark, and mutant blind females are not yet available. If they did court freely in the dark it is possible that the females, like simulans females,

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would be reticent to mate. Similarly, Pinsker and Doschek (1980) have shown that although D. subobscura males are largely responsible for the lack of mating in the dark in this species, the females are also partially light-dependent. Nevertheless, the absence of these visual displays, rowing and scissoring, from about half the successful courtships observed indicates that they are not of overriding importance.

Secondly, the sound frequency of the sine song component of the mauritiana males' wing vibration is higher than that of simulans in this study, but not in Cowling and Burnet's (1981). Again the significance of this is questioned by the observation that sine song is not present in every successful courtship.

Thirdly, in mauritiana pulse song the parameter usually thought to be most important, i.e. the inter-pulse interval, is extremely variable. Although there has been some debate as to the relative importance of auditory and olfactory stimuli in Drosophila courtship (see Averhoff et al. 1979), females are capable of receiving auditory stimuli (Ewing 1978) and they do appear to play a role (Schilcher 1976b; Ikeda et al. 1981). Nevertheless, the variable pulse song of mauritiana males may no longer be used by the female as a stimulus during courtship. Although the observation that wingless mauritiana males are still at a disadvantage in the dark suggests that non-visual stimuli are important, the females may in the dark depend on olfactory stimuli, or even the sine song. Also, the wingless males may not be stimulating each other to court (see Schilcher 1976a). Playback experiments such as those of Schilcher (1976b) are needed to settle this question. Until then Cowling and Burnet's (1981) suggestion that mauritiana females

recognise the modal ipi (which is considerably lower than the mean, and similar to that of melanogaster) must remain speculation. Bennet-Clark and Leroy's (1978) suggestion that the females of Z. mascariensis (the males of which also have an extremely variable courtship song) recognise variable songs as such is doubted because present results indicate that non-visual stimuli from the males' wings are unimportant. Applied to D. mauritiana this idea also remains speculative.

Fourthly, mauritiana males' attempts at copulation are quicker than those of simulans and melanogaster. This appeared to be the immediate reason for the ease with which mauritiana males mated simulans females, i.e. they simply took the females by surprise, whereafter the females always objected. The only other report of a similar phenomenon involves D. subobscura males courting D. pseudoobscura and D. persimilis females (Wallace and Dobzhansky 1946). Why the females subsequently objected so vigorously has not been resolved. As a working hypothesis, the actual speed of the copulation attempts may be the crucial difference. Removal of a numb of potentially different stimuli such as those provided by the males' wings, foretarsi, sexcombs, proboscides and even the posterior lobes of the genitalia, or those received by the females' antennae did not remove the reason for the females' objection. Nevertheless, a combination of these or still other stimuli, such as other genital structures or seminal fluids, may be involved. Similarly, the reason for the consistent rejection of simulans males by mauritiana females may either simply be the slowness of attempts at copulation or may involve a number of

stimuli.

A search for differences in a single crucial stimulus is not completely unreasonable, especially when examining closely related species, since Mayr (1950) and Ehrman (1959) found that removal of the females' antennae greatly increased interspecific insemination between D. pseudoobscura and D. persimilis. Nevertheless, no single crucial difference was found for any of the species pairs examined here, and a brief attempt at multi-stimulus removal using foretarsiless and wingless melanogaster males with antennaeless mauritiana females was also unsuccessful, i.e. there was no increase in interspecific mating (see Table 22).

Finally, in agreement with Watanabe and Kawanishi (1979), of the three species studied, mauritiana females are least inclined to mate interspecifically, whatever the reason. They also were most sensitive to removal of stimuli in intraspecific courtships. Together with the increased specificity of mauritiana male responses discussed earlier, this indicates that, in addition to the changes in mauritiana courtship, mauritiana mating responses are generally at least as specific as those of simulans and melanogaster.

4.4b Evolution

In addition to the above courtship changes, mauritiana has diverged from simulans with respect to wingbeat frequency (this study), male genitalia (Tsacas and David 1974), some allozymes (Eisses et al. 1979), DNA composition (Barnes et al. 1978) and some genetic factors causing male hybrid sterility (David et al. 1976).

Excluding models not involving some allopatric differentiation

(see Chapter 1), there are two broad models of speciation which might explain this divergence. The first holds that colonization of islands such as Mauritius is a rare event, and that mauritiana evolved in total isolation (allopatry) from an ancient colonization by, probably, a single gravid simulans female. Reunion would have been colonized independently, and perhaps more recently, by simulans and melanogaster without any speciation taking place, but neither have subsequently colonized Mauritius. This is the sort of explanation offered by David and Tsacas (1975), after consideration of distribution patterns on these and other islands.

The second model holds that colonization is not so rare an event and that after an initial period of isolation during which some genetic divergence took place, particularly with regard to subsequent male hybrid sterility, repeated re-colonizations by simulans flies, while never successful for long, led to reinforcement of behavioural isolating mechanisms between the two populations in the form of changed mauritiana courtship. Although this model avoids the two major problems usually associated with reinforcement models of speciation as discussed in Chapter 1, in this case there are two reasons for doubting its likelihood. Firstly, the female hybrids are fertile and backcross progeny from these show no evidence of genetic breakdown. Hence the selection pressure for reinforcement would be greatly reduced, perhaps by half, from the situation usually modelled where all hybrids are sterile. This would also lead to considerable introgression which would prevent further divergence (see Chapter 1 and Futuyma 1979, p. 407). Secondly, another explanation is needed for the absence of melanogaster from

Mauritius and of mauritiana from Reunion. Island-dependent ecological competitive exclusion does not provide a compelling explanation. Hence I adopt the first model of speciation in total allopatry as being the most reasonable explanation of the origin of mauritiana.

D. mauritiana therefore provides a test of the prediction, presented in the Introduction, that isolated species should have relaxed ethological isolating mechanisms. The variable pulse song of mauritiana appears to support this prediction. However, it has yet to be established that mauritiana females still use this pulse song for mate recognition. It is worth noting that Z. mascariensis, which also has a variable courtship song (Bennet-Clark and Leroy 1978), is endemic to Mauritius and Reunion, and is sympatric with a sibling species, Z. tuberculatus, on the latter (David and Tsacas 1975; Tsacas et al. 1977). The strains used by Bennet-Clark and Leroy (1978) (see Bennet-Clark et al. 1980) and in this study come from Mauritius. Examination of the Reunion strain would provide another test. If its courtship song is less variable the isolation concept would be supported, and present results indicating that the courtship song is no longer used would be questioned. A major advantage of working with Drosophila is that, in addition to measuring the variability of courtship parameters, the actual specificity of mating responses can be ascertained. As discussed above, both males and females of mauritiana are generally more specific in their mating responses than are either simulans or melanogaster, two closely related species which occur sympatrically worldwide. This clearly does not support the prediction from the isolation

concept of species, and instead agrees with the alternative view of Paterson (1978, 1980, 1981, 1982) whose recognition concept of species predicts that isolated species should have mating responses which are as specific as those of any other species. The specificity of mauritiana mating behaviour also supports the contention that mating behaviour can diverge in allopatry without loss of specificity.

Finally, as Watanabe and Kawanishi (1979) reported, mauritiana, simulans and melanogaster exhibit asymmetrical interspecific mating propensities. This is particularly clear when mauritiana is involved. Their explanation of this in terms of reinforcement in complete sympatry is however untenable. Similarly, Wasserman and Koepfer's (1980) alternative character displacement explanation is considered unlikely, as argued above. Kaneshiro's (1976, 1980) original explanation involved loss of aspects of the courtship. D. mauritiana appears instead to have added to its courtship, or at most to have changed to a more light-dependent courtship. Furthermore, the proximal cause of the asymmetry (the rapid copulation attempts of D. mauritiana males) is unrelated to the complexity of the courtship. Clearly, as Wasserman and Koepfer (1980) urge, individual cases must be examined separately, and it is improbable that such asymmetries, if they reflect phylogeny, do so for any particular reason.

Chapter 5. SPECIFIC-MATE RECOGNITION AND MECHANICAL ISOLATION IN ENALLAGMA DAMSELFLIES

5.1 Introduction

The concept of mechanical isolation, first mooted by Dufour (1844), became popular with the development of the theory of speciation by reinforcement. Very little empirical evidence for this phenomenon was produced however, and as a result, both Mayr (1963, 1970) and Dobzhansky (1970) concluded that mechanical isolation was relatively unimportant in the maintenance of species integrity. It remains a possibility however, and is still discussed by recent writers of books on speciation and evolution (e.g. Maynard Smith 1975; Dobzhansky et al. 1977; Grant 1977; Stansfield 1977; White 1978, Futuyma 1979).

The mating behaviour of many Odonata has previously been explained in terms of mechanical isolation (Williamson 1906; Johnson 1962a; Paulson 1974). This group of insects is particularly suited to this explanation because their copulation involves two separate points of contact. When a male encounters a female, he grasps her thorax with his legs and then clasps the female's prothorax with his anal appendages, thus achieving the tandem position (Fig. 11A). The second stage of mating involves female recognition, when she co-operates with the male in bending her abdomen under him to make contact with his accessory genitalia on the second abdominal segment, and so achieves the copula position (Fig. 11B). Mechanical isolation may operate at either stage, but the attainment of tandem is usually thought to be the more probable site.

The best evidence for this theory was provided by Paulson (1974). He pinned live females of a number of species of

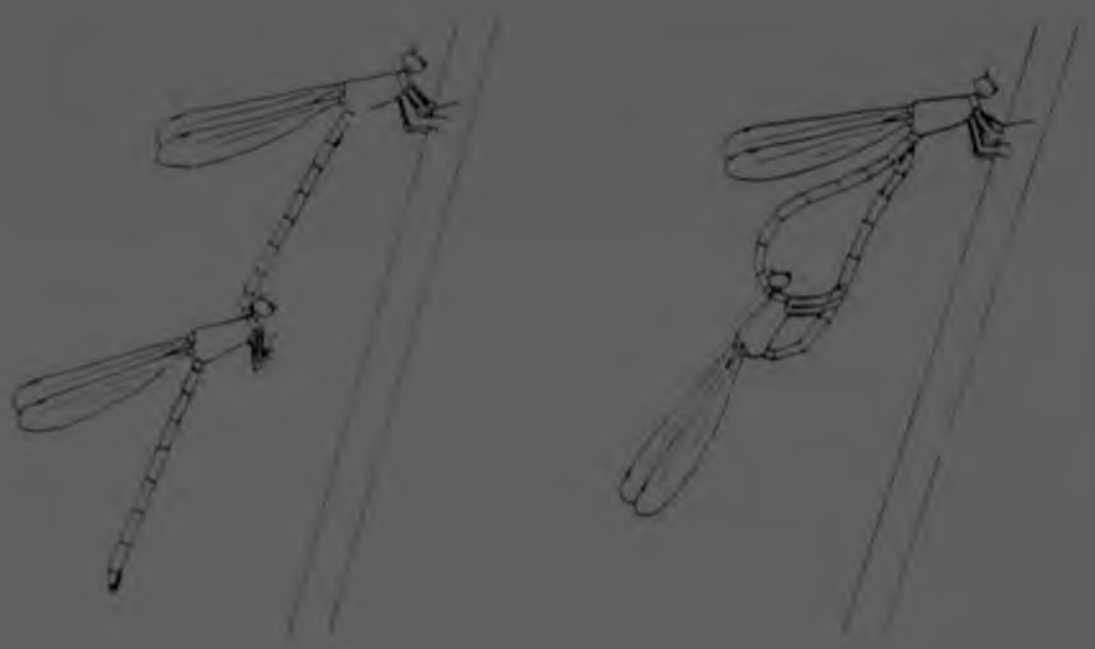

Figure 11. Copulation in Zygoptera. A (left) - The male clasps the female with his anal appendages on her prothorax, yielding the tandem position. B (right) - The female bends her abdomen under the male to contact his accessory genitalia, yielding the copula position.

coenagrionid damselflies to sedge stems and presented them to males of the various species. He then scored whether the male approached and attempted to clasp the female, and whether he was successful in clasping her. The degree of mechanical isolation could then be expressed as the proportion of attempted tandem linkages which were unsuccessful. Of the seven intrageneric species-pairs (in which the males almost always attempted to clasp the female), four showed nearly complete, two partial and one very little mechanical isolation. Thus, for most of these species, reproductive isolation is normally maintained by the inability of males to clasp non-conspecific females with their anal appendages, presumably because their differently



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shaped and sized appendages are not compatible with the thoracic structures of the non-conspecific females.

Since Paulson only discussed how males are reproductively isolated from non-conspecific females, he did not offer an explanation for how the integrity of the species-pair which showed very little mechanical isolation is maintained in the sympatric situation he studied. This might be explained by the work of Loibl (1958) and Krieger and Krieger-Loibl (1958).



Figure 12. A pair of Enallagma glaucum ovipositing in tandem.

They, especially Loibl, concluded that in the genera Lestes (Lestidae) and Ischnura (Coenagrionidae), females recognised conspecific males by the tactile stimulation of the anal appendages when clasped in tandem. They observed that in both genera males would readily attempt [illegible]netimes attain tandem linkage with non-conspecific females. These females then refused

to co-operate in achieving copulation, and were eventually released. Furthermore, Loibl found that in Lestes, females would not co-operate when clasped by males whose inferior appendages had been altered, implying that it was the shape of these that the females used to recognise conspecific males.

Demonstration of the existence of such a tactile recognition system might provide an alternative evolutionary explanation of the rest of Paulson's (1974) results, and would considerably improve our understanding of odorate SMRS's, so I endeavoured to identify the site and method of this recognition system in females of the genus Enallagma (Fig. 12) the group on which Paulson did most of his work.

5.2 Materials and Met'

The first problem was to identify a p s ble site for tactile recognition on the female's thorax. Previous attempts to study the tandem linkage points in Zygoptera have not yielded detailed information (Williamson 1906; Tillyard 1917). I eventually succeeded by carefully capturing tandem pairs, by hand or with a net, immediately before or during copulation and dropping them into liquid nitrogen. Some were frozen in the tandem position. These were then air-dried, coated with gold-palladium, and examined with a scanning electron microscope. This study was only feasible with the locally occurring and abundant species, E. glaucum.

A comparative study was then done, using the scanning electron microscope, of the male anal appendages and female thoracic structures of six Southern African species. Between two and five specimens of each sex and species were examined. The specimens of E. glaucum Burmeister, E. rotundipenne Ris and E. sapphirina Pinhey were from Johannesburg (Transvaal), E. nigridorsum Selys

from Kranskop (Natal), E. sinuatum Ris from Karkloof Falls (Natal, and E. subfurcatum Selys from Umtali (Zimbabwe).

Finally, an experiment similar to that of Loibl (1958) was conducted using a population of E. glaucum at man-made ponds on the campus of the University of the Witwatersrand. Males were caught, marked with a spot of enamel paint and the tips of either their superior or inferior appendages were snipped off with the fine scissors fashioned from watchmaker's forceps. Most males, on being returned to the pool, seemed unaffected by the operation. They readily chased and caught females, and were fully capable of clasping and holding them. The outcomes of all tandem linkages (by normal and operated males) were noted and classed according to whether the female co-operated in copulating with the male or not. These observations were conducted from 10-00 to 13-00 h for twenty-one days (15.11.79 - 6.12.79).

5.3 Results

Fig. 13 shows the tandem linkage in E. glaucum. The male's anal appendages are splayed out laterally, so that it is their inner surfaces which make contact with the female's thorax. The inferior appendages press down on the posterior dorsal half of the prothorax, while the superiors lie on the mesostigmal plates (laminae). The superiors have large hooks (Fig. 14) which fit under the posterior flange of the prothorax. This confirms Williamson's (1906) suggestion that the clasping action is accomplished by the inferiors and the hooks of the superiors acting in opposition to each other on the prothorax in a pincer-like fashion.

Possible sites for tactile recognition on the female would be the dorsum of the prothorax or the mesostigmal plates. When



Figure 13. A lateral view of the tandem linkage points in E. glaucum. The male's inferior anal appendage (right) contacts the female's prothorax, which the superior appendage (left) lies on the mesostigmal plate (50X).

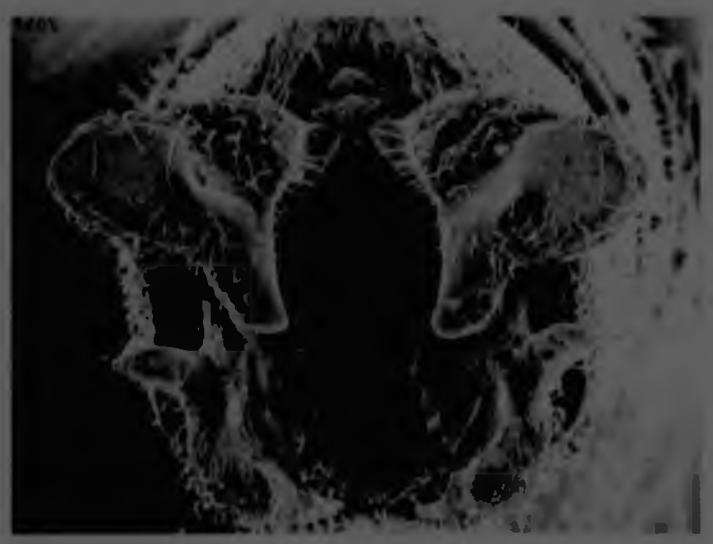

Figure 14. A posterior view of the male anal appendages of E. glaucum splayed out laterally as they are while clasping the female. The superiors are above (75X).

these areas were examined, no obvious sensilla other than long hairs were found on the prothorax, but the mesostigmal plates were found to be groove-shaped and to contain short peg-like sensilla (Fig. 15) which appear to be basiconica mechano-receptors (Fig. 16) (Schwartzkopf 1974; McIver 1975). These short sensilla, spread over the groove, would be ideally suited to the recognition of the appropriately convex inner surface of the male superior appendage.



Figure 15. A dorsal view of the right mesostigmal groove with sensilla of an E. glaucum female (200X).

The comparative study yielded support for this hypothesis. The inner surfaces, as well as the general shape and size, of the superior appendages differ markedly in the six species studied (Fig. 17). The inferior appendages in contrast are all very similar. This pattern is also present in other Enallagma species (e.g. Johnson and Westfall 1970; Johnson 1972). In each species there is also a ventral projection on the superior



Figure 16. A single sensillum from an E. subfurcatum female (2300X).

appendage which presumably functions in clasping the female, in a manner similar to the hook in E. glaucum.

In each species, the females have modified mesostigmal plates with sensilla (Fig. 17). The general shape of the mesostigmal grooves and the distribution of their sensilla correspond appropriately to the shapes of the inner surfaces of the conspecific male's superior appendages (Fig. 17). For example, E. subfurcatum males have long superior appendages while the female's groove is shallow and the sensilla are distributed along its entire length. E. sapphirina males in contrast have very short superiors with a pronounced inner bulge while the female grooves are deepest nearest the midplane of the thorax, with the sensilla concentrated here. E.

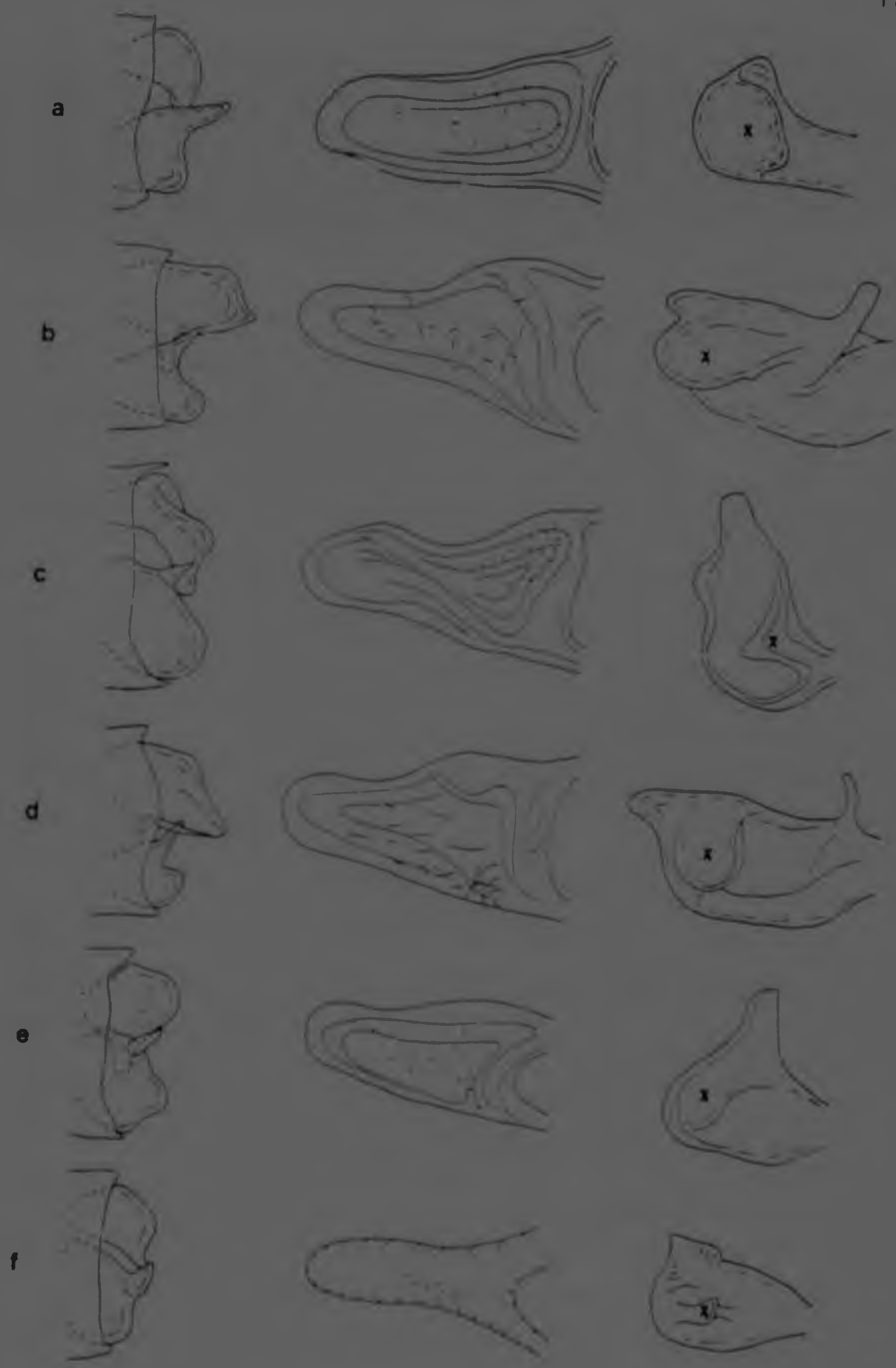

Figure 17. Male anal appendages and female mesostigmal grooves of six species of Enallagma. The first column presents lateral

views of the male anal appendages (± 35X), the second the left mesostigmal grooves, with sensilla, of the females (± 85X), and the third the inner surfaces of the male superior appendages (± 70X). The "X" marks the area believed to fit into the female groove. The species are (a) E. sapphirina, (b) E. subfurcatum, (c) E. sinuatum, (d) E. rotundipenne. (e) E. glaucum and (f) E. nigridorsum. The drawings were traced from scanning electron micrographs.

nigridorsum is an extreme example, since the male superiors are very small and have a sharp ridge on their inner surfaces. The females have mesostigmal plates developed into ridges, rather than grooves, on which the few sensilla stand, thus complementing the males' small superiors. Although the sensilla in some species are quite long, I believe they perform the same tactile recognition function.

Perhaps because of developmental constraints, the males of each species also have mesostigmal grooves similar in shape to those of the conspecific female, and containing sensilla (although always fewer than in the female). In each species, however, the males have a ridge or flange developed on the edge of the grooves nearest the mid-dorsal line of the thorax. This structure is most dramatic in E. rotundipenne and E. subfurcatum (e.g. Fig. 18). This might prevent males from clasping each other. Such unsuccessful attempts were occasionally noticed in E. glaucum.

The results of the experiment on E. glaucum using males with damaged anal appendages are presented in Table 26. Most females mated readily and quickly with normal males, as would

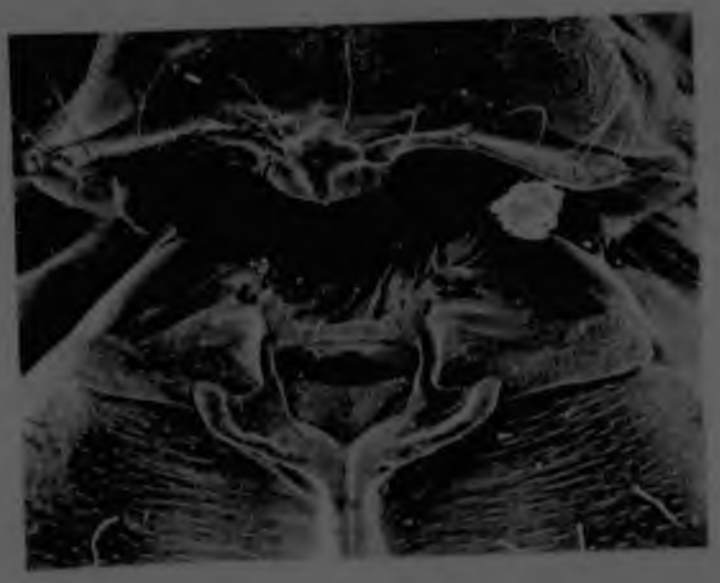

Figure 18. A dorsal view of a male E. rotundipenne showing the large flanges on the midline edges of the mesostigmal grooves (50X).

be expected. Those that refused to co-operate with normal males appeared to be either immature or had recently mated with other males. Usually these females would actively reject and avoid capture by males, but were occasionally caught. The males with the tips of their inferiors removed yielded results which were almost identical to those for normal males. This supports the hypothesis that the inferiors only function in clasping the female, and also provides a control for the experimental removal of the tips of the superiors.

When the superiors were damaged, most females refused to co-operate with the damaged males, for example, they would grasp the substrate or simply hang from the males' abdomens, and were eventually released. This is similar to the behaviour reported when females are clasped by non-conspecific males (Loibl 1958; Krieger and Krieger-Loibl 1958; Tennessen 1975).

Table 26. The numbers of females which mated or refused to mate with males whose anal appendages had been damaged.

Female Response	Male Appendages Damaged		
	Neither	Inferiors	Superiors
Refused	4	4	49
Mated	25	26	12

Those that did mate usually did so only after a long period in tandem with the male. The males were examined at the end of the experiment, and all were damaged to some extent. However, the snipping operation is rather difficult and imprecise, so some males had less of the appendages removed that did others. Furthermore, only the tips of the appendages could be removed and so, since I believe that in this species most of the length is required to stimulate all the sensilla, only part of the male signal was removed. It is possible that some females would accept these only partially impaired male signals. Overall, the results of this experiment are in accord with the hypothesis that there is tactile recognition of the shape of the male's superior anal appendages.

I attempted to complement this experiment by removing or inactivating the female's sensilla, but was unsuccessful because the small size of the mesostigmal groove and the sensilla made it technically very difficult.

5.4 Discussion

The work of Loibl (1958) and Krieger and Krieger-Loibl (1958)

is confirmed and extended with these findings in Enallagma. Firstly, the females' mesostigmal plates are developed into tactile recognition sites which, during tandem, would be stimulated by the inner surfaces of the males' superior anal appendages. Secondly, there is a good correspondence between the shapes and sizes of the male superior anal appendages of six Southern African species and the shapes, sizes and distribution of sensilla of the recognition of their conspecific females. Thirdly, in one of the species studied, females usually refused to mate with males which had had part of their superior anal appendages removed. I later discovered (see Corbet 1980) that Tennessen (1975) did similar work on two North American species of Enallagma, and suggested a similar tactile recognition system.

This type of recognition system probably evolved along with the evolution of the unique mode of copulation in the Odonata (see Fraser 1939). Similar tactile recognition systems will therefore probably be found in many other odonates. Some species, however, have secondarily developed visual recognition systems. For example, Pajunen (1964) found that the superior anal appendages of two Leucorrhinia (Anisoptera) species were not important in species recognition, but could not examine whether the inferior appendages were involved. However, the females of these species seemed to recognise conspecific males visually prior to being clasped in tandem. A number of other odonates also appear to have female visual recognition of conspecific males before tandem (Jacobs 1955; Bucholtz 1956; Furtado 1975; Robey 1975; Williams 1977). Species of two families, the Calopterygidae (Johnson 1962b ; Pajunen 1966;

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Waage 1973) and the Chlorocyphidae (Consiglio 1974; Chapter 6), have complex male courtship displays which probably involve female visual recognition of conspecific males prior to tandem. In all these species, a tactile recognition system during tandem may either still be present and functional, or have been lost.

To summarize, odonate mate recognition systems consist of an initial visual recognition step made by the male as he responds to and approaches the female. As noted earlier, this is often inaccurate within genera (e.g. Johnson 1962a; Paulson 1974), but is sometimes more precise (e.g. Waage 1975). This is followed by either a visual recognition step by the female before she is clasped in tandem, or a tactile recognition step involving the male's anal appendages once she is clasped in tandem, or possibly both.

As described earlier, Paulson (1974) found that in several North American Enallagma species, the males were unable to achieve attempted tandems with non-conspecific females. Effectively therefore, they are mechanically isolated. Paulson considered the differences in the structures of the male anal appendages and female thoraces responsible for this to "...have evolved in response to the advantage of reproductive isolation", i.e. to be mechanical isolating mechanisms. As discussed in Chapter 1, this is considered to be unlikely. Other authors (e.g. Mayr 1970) might argue that they originated fortuitously in allopatry. While this is entirely plausible, and may be true for other groups as well, in this case there is a third possibility.

In the light of the existence of a tactile recognition system involving these structures in Enallagma, it is argued that

the mechanical isolation observed by Paulson is a secondary, incidental consequence of changes in such a system in allopatry. Any new species must have male anal appendages and female thoracic structures differently shaped from other species, i.e. a new tactile recognition system, if it is to co-exist in sympatry with them. If such differences are great, then incidentally, the males may not be able to clasp non-conspecific females. How such changes in recognition systems take place in allopatry will be considered in Chapter 7.

Chapter 6. MATING BEHAVIOUR AND ITS RELATIONSHIP TO TERRITORIALITY IN SOME CHLOROCYPHID DAMSELFLIES

6.1 Introduction

The study of odonate mating behaviour has involved mostly Northern Hemisphere and temporal region species, for which the mating behaviour of at least some species representing most major families has been described (Corbet 1980; Chapter 5). Perhaps because the Chlorocyphidae occur only in Africa and tropical Asia, to the best of my knowledge, the courtships of only three species of this large family have only briefly been described. Carpenter (1928), Pinhey (1951, 1968) and Consiglio (1974) described the display of the white tibial fronts in Platycypha caligata, Neville (1960) described a display involving the last few segments of the abdomen in Chlorocypha glauca, and Fraser (1934) (quoted in Paulson 1981) described a Rhinocypha sp. courtship as involving display of the hindwings and tibiae. Chlorocyphid mating behaviour is of additional interest because they are perhaps related to the well-studied Calopterygidae (Fraser 1954). Indeed, as described here, Chlorocyphid courtship is at least as dramatic and complex as that of the Calopterygidae, which makes it of interest in attempts to explain the evolution of such courtship.

Here I describe in some detail the mating behaviour of the fairly common species Platycypha caligata (Selys). This is followed by a comparison with the apparently closely related species P. fitzsimonsi (Pinhey), including an attempt to establish which differences in their SMRS's allow their sympatric coexistence. Observations on another species, Chlorocypha consueta (Karsch), are then reported and the courtship display

of Thinocypha unimaculata Selys is described from a brief film made by Dr. J.M. van Brink. Finally, from the work on P. caligata and with reference to the other species, a theory for the origin of such courtship in the Odonata is developed.

6.2 Materials and Methods

The study area for P. caligata was in Tonquani Gorge in the Magaliesberg (Transvaal, 25°51'S, 27°30'E). Most of the observations were made along a 40m section of the stream in a fairly open part of the gorge. The stream is small (approximately 3 m wide), rocky and swift-flowing with no emergent vegetation. The banks are covered with natural riverine forest which shades the stream during early morning and late afternoon. Observations were made during the two summer periods of 1977 (5, 12, 19 and 27 February; 19, 20, 26, and 27 March; and 7 - 23 December). Observations were recorded continuously for the period that individuals were active at the stream, and totalled 61 h. Many individuals were marked with unique combinations of "Humbrol" enamel paint dots on the thorax and/or abdomen. Most individuals, upon release after marking, returned to the stream within minutes, and their behaviour was not noticeably different from that of unmarked ones.

P. fitzsimonsi was observed on the Umzimkulwana River in Oribi Gorge (Natal, 30°42'S,30°16'E), where it occurs sympatrically with P. caligata, on 28-30 March 1978 and 28-31 December 1978, and at a stream near the Royal Natal National Park (R.N.N.P.) (Natal, 28°39'S, 29°02'E), where it occurs alone, on 20, 21 January 1981. At Oribi Gorge females were individually marked as above. At the R.N.N.P. the meso- and metatibiae of males

were painted white anteriorly.

C. consueta was observed on the Nyamataka River near the Vumba Mountains in Zimbabwe ($19^{o}12'S$, $32^{o}46'E$) on 9, 12 December 1980. Dr. J.M. van Brink observed and filmed R. unimaculata on the Godawari River, near the Royal Botanical Gardens in the Katmandu Valley, Nepal on 27 May 1973.

6.3 Results - Platycypha caligata

6.3a Description

Chlorocyphids are atypically robust damselflies (Zygoptera) whose flight and general behaviour are correspondingly more



Figure 19. A Platycypha caligata male. The white fronts of the expanded tibiae can be seen below and beyond the thorax.

more vigorous than that of other Zygoptera. They are usually highly sexually dimorphic in colour. P. caligata males have bright blue dorsal surfaces of abdominal segments 3 - 10, and

laterally expanded tibiae which are brightly coloured, white anteriorly and red posteriorly (Fig. 19). The females lack the expanded tibiae and are cryptically coloured with black and light brown. The species is found throughout Africa south of the Sahara. On the South African highveld it is restricted to streams in mountain ranges such as the Magaliesberg and Drakensberg.

6.3b Territorial Behaviour

Males descended first from trees along the stream (where the damselflies presumably spent the nights, early mornings and late afternoons) soon after direct sunlight fell on the stream. They were active only if there was direct sunlight, and did not appear on cloudy or rainy days. Since the sunlight left the stream in the early afternoon, they were only reproductively active for four or five h over midday. It is assumed that most reproductive activity takes place at the stream (see Waage 1973; Corbet 1980).

The males were considered to be territorial in that they often remained in, or returned regularly to, the same area and maintained exclusive use of this area by active exclusion of intruders. Territories were centred around oviposition sites (driftwood or roots of trees which are partially submerged), with a perch (a rock or stick in the stream) in the sunlight nearby. They varied in size from circles 0.5 m in diameter to large ar (4 m x 3 m) depending on the proximity of the perch to the oviposition site (which were occasionally the same), and the l density of males.

Oviposition sites were mapped along the 40 m section of the stream. During December, 57 males were marked and their positions

along the stream and territorial status were noted three times each day for eleven days. Of these 47 were present at the stream for a mean of 3.7 ± 2.3 (S.D.) days (range 1 - 9), but were territorial for only 2.0 ± 2.2 days (range 0 - 6). These are clearly underestimates since some of the males when marked were quite old, and the survey was limited to the 40 m section of the stream for only eleven days. Nevertheless they show that there were about equal numbers of territorial and non-territorial males ("territorial" indicating only control of a territory, not a genotype or mating strategy), with frequent changes in territory control. For example, no male was recorded as being in control of a particular territory for more than three consecutive days. During February and March, the density of males was about half that during December, yet there were still some non-territorial males.

Non-territorial males fly slowly along the stream, apparently inspecting the banks, and particularly crannies between rocks where driftwood is often trapped, for possible oviposition sites. These are then examined closely while hovering. In what appears to be a further inspection, the male may then land on the site, and "tread" (i.e. lifting and replacing one or two legs at a time). Territorial males return immediately each day to their previous oviposition site and territory. They regularly make patrol flights of the territory, visually inspect their oviposition sites, and occasionally tread on them.

The territorial interactions consist of an hierarchical series of behavioural components and displays during which the males were never seen to make physical contact. Although

these interactions were continually observed, no quantitative data were collected, and the behaviours involved will simply be described.



Figure 20. The white flash display. A territorial male (above) challenges an intruder, which is on an oviposition site (a stick dipping into the water), with a flash display of the white fronts of the tibiae.

An intruder flying past is simply followed till it is out of the territory. An intruder which perches in the territory is challenged by the resident who darts at the intruder and displays the white fronts of his tibiae in a quick flash display (Fig. 20). The intruder usually flies up immediately, but sometimes, especially while treading on the oviposition site, responds by wing-spreading, i.e. spreading his wings apart and raising his abdomen, a response observed in many Odonata (e.g. Pajunen 1963; Bick 1972). The resident then repeatedly challenges him till he flies.



Figure 21. The red flash display. An atypical usage of this display in challenging a perched intruder. The waggling of the body can be seen.

Once in the air, the resident, waggling his abdomen sideways, flies at the intruder and gives a red flash display by bending his tibiae upwards through 180^{0} (Fig. 21). The intruder joins in this "waggle" flight, and then both retreat and fly at each other again. These flights end with progressively higher upward flights before the two drop back and start again, and last about 3 s each. The upward component at the end of these flights soon develops into a "parallel" flight in which the two males hover side-by-side, either head-to-head or head-to-tail, and about 4 - 6 cm apart in short, jittery hops, while gaining height. After each parallel flight the two drop back down and start again. These flights gain height progressively, and after a while the waggle component at the start is largely dispensed with. Each parallel flight lasts 5 - 20 s.

The parallel flight soon becomes more ordered, with the two facing in the same direction in the upper part of the flight. This is preparatory to the next component, in which the two males, at the end of the parallel flight (about 3 - 7 m above the stream), fly off rapidly in a straight line side-by-side at an angle of about 30° to the horizontal. After this "long flight" both males return separately to the site and start again.

On only two occasions was a further component observed. The resident, after hours of these interactions, suddenly started chasing the intruder wildly with continuous displays of the white tibiae. In both cases the resident eventually retired, apparently exhausted, and the intruder took over the territory.

These interactions could be brief and only go as far as the waggle flight, or sometimes last for more than a day before one male retired. Males on adjacent territories seldom engaged in these long interactions, but made alternating pursuit flights across the border between their territories. When three or more males interacted they simply followed each other in a small circle until one or more retired. The remaining pair would then start the interactions described above.

The blue abdomens of the males were thought to be important in male - male recognition. This was tested by a brief experiment using simple models. Small sticks approximately the size of the males were painted blue, red , white and brown on one dorsal half, and dangled on cotton in front of territorial males. Each was tested twice on three different males. The blue model always elicited waggle flights, with clear red flash displays. The other models were simply avoided by the males. Dead males dangled near territorial males similarly elicited attempts to

initiate a waggle flight. In another brief experiment, females' abdomens were carefully painted blue. Males sometimes reacted to these females as if they were males, but always eventually courted and sometimes copulated with them. Obviously other morphological, colour or behavioural characteristics are also important in male recognition of males and females.

6.3c Mating Behaviour

During December, 43 females were marked and censused with the males. Of these only 22 were seen again in the next six days. Their occurence was both spatially and temporally sporadic. The females readily moved up and down the stream while males were often localized in particular areas, even if not territorial there.

The females, on arriving at the stream, perch on plants along the banks, from which they forage. These foraging females are largely ignored by the males below. Eventually, they descend to, and fly slowly along the stream. When a female flies through a male's territory, he darts out and intercepts her. He seems to give a brief display of his white tibiae (this was not always clearly seen), and circles the female who slows down and hovers. He then turns and flies slowly back to his oviposition site in his territory. During this flight he hangs his abdomen down and swings it from side to side, clearly displaying its blue dorsal surface. The female follows closely to the site, at which the male turns and starts courting from behind or alongside it. The female lands on the site and makes oviposition movements on it. She continually gives a wing-spreading display, but without the raised abdomen, while the male courts her. The courtship is a dramatic display of the white tibia which are extended below the thorax and vibrated rapidly (Fig. 22), while



Figure 22. The male's courtship display. The male (above) is displaying the white fronts of his tibiae to the female on the oviposition site. She is making probing oviposition movements on the site while wing-spreading.

the male describes an arc around the female, always facing her and 1 - 6 cm from her wingtips (Fig. 23).

The courtship lasts from a few seconds to more than a minute, depending on the female. In a successful mating, the female flies slowly up from the site and perches, with her wings closed above her abdomen, on a nearby rock or plant. The male follows closely, lands on her thorax, and achieves the tandem position. The male spreads his wings, and, while vibrating them, lifts the female off the substrate. She then bends her abdomen under his to connect genitalia in the copula position. The copula position is attained very quickly and accurately. As a result it was not always clear whether the male transferred sperm from his genital pore to his accessory genitalia while in tandem. This was

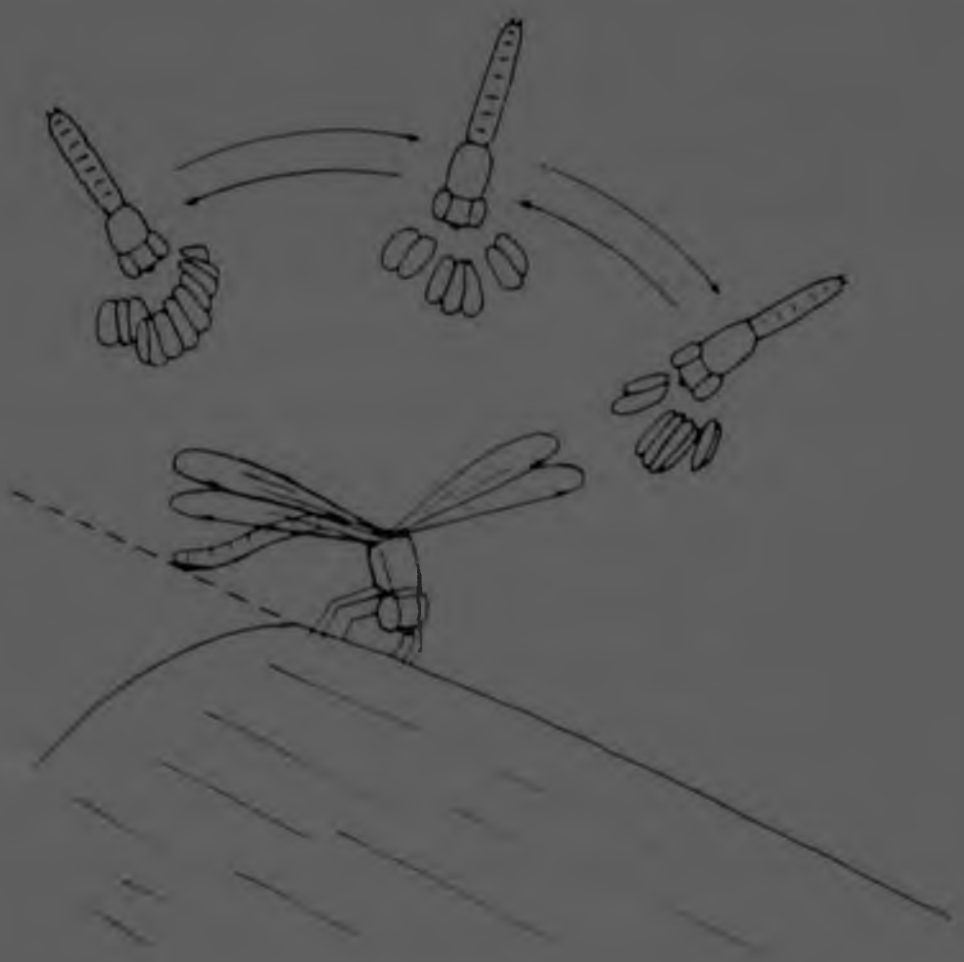

Figure 23. The male's courtship display. The male describes an arc around the female. This drawing was traced from three superimposed 8 mm movie frames such as Fig. 22.

certainly sometimes the case, as it seems to be in all other Zygoptera (Bick and Bick 1965). The average length of 19 timed copulations was 57 ± 17s (range 30-91). During copulation the male "pumps" his abdomen up and down, a behaviour apparently common to most Odonata, which Waage (1979b) and Miller and Miller (1981) have suggested may be involved in the removal of sperm from previous matings. The end of copulation and release of genitalia is again associated with the male spreading and vibrating his wings. The male keeps the female in tandem, and flies with her to his site where he releases her. He again

courts her from behind or alongside the site until she lands and starts ovipositing. He then returns to his normal perch.

No distinct guarding behaviour, as found in many other odonates (Corbet 1980), was observed, since subsequent interactions with other males appeared to be the same as for normal territorial defence, excepting that the intruder, upon seeing a female ovipositing on the site, appeared to be more excited and persistent. Males showed no refractory period after mating as sometimes observed in Calopterx (e.g. Waage (1979c), but not Alcock (1979)), i.e. they would readily mate again immediately. As a result males often had more than one female ovipositing at the same time. A female would oviposit at a particular site for a few seconds to more than an hour. Females too would mate repeatedly with different males, often many times a day (up to five copulations per female per day were observed).

6.3d Pair-Formation Sequences

The above is a description of what appears to be the basic sequence of events in a successful mating. There are however many variations on this basic sequence. Following Waage (1973), these various interactions can be subdivided into three categories of encounter :-

type I - a territorial male intercepts a female,

type II - a territorial or non-territorial male finds a female ovipositing,

type III - a territorial or non-territorial male finds a perched female.

All interactions from February, March and December have been included. Fig. 24 is a flow-chart of the type I interactions. The central sequence is that described above. Males were observed

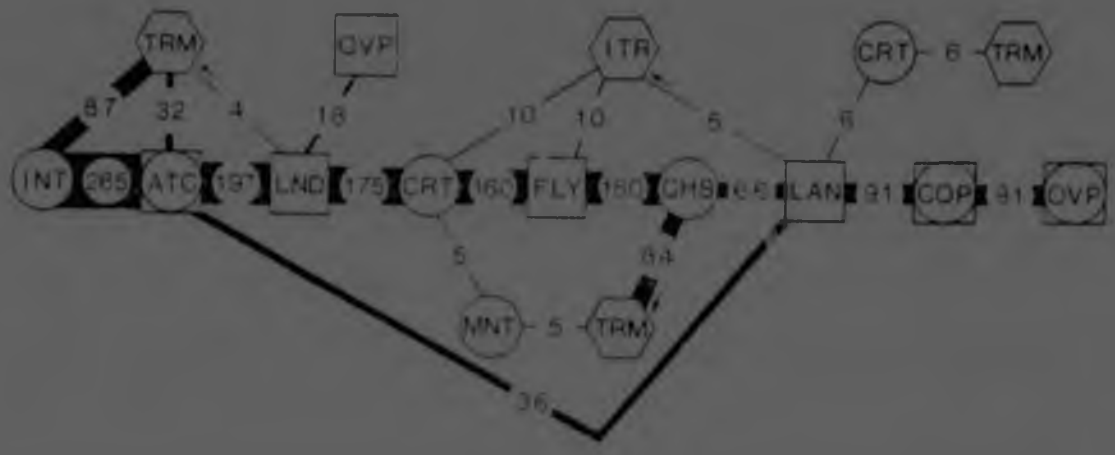

Figure 24. Flow chart of type I interactions. Key to Figures 24, 25 and 26 :- Circles indicate male actions and squares female actions. Hexagons indicate terminations of interactions other than oviposition. Except where indicated, the flow is from left to right. The numbers on the lines and their relative thickness indicate the number and percentage of interactions following a particular sequence. Meanings of the abbreviations: ATC - attraction to site; ATT - female attacks male; CHS - male chases female; COP - copulation; CRT - courtship; FLY - female flies; INT - interception; ITR - interference; LAN - female lands on perch; LND - female lands on site; LVE - male leaves female; MNT - male mounts female; OVP - oviposition; RST - female resists; TRM - termination.

attempting to intercept 352 females. Of these, 87 were immediately unsuccessful, because the female either ignored the male (because she was escaping another, or feeding), or stopped following him before they reached the oviposition site (often because she lost him, or was interfered with by another male). Of those successfully attracted, 32 did not land on the site, but flew off and evaded the male (decamped). Of these seven seemed to result from the

female being unable to land because the male courted her incorrectly, i.e. in front of, instead of alongside or behind the site (usually because the site was in too enclosed an area). Another five seemed to result from there being no clear site to which the male was attempting to attract the female. Males would occasionally defend such areas, perhaps because of the intense competition for real sites. The cause of the remaining 20 failures was unclear.

The next variation, involving 36 females, was the only other successful sequence in type I interactions. These females did not land on the site, but after hovering near it briefly, flew slowly up to a perch and copulated. The reasons for this variation being successful will be discussed later.

Following Fig. 2[illegible], the next (4 observations), sixth (5 observations) and last (6 observations) variations, although uncommon, demonstrate that to be successful, the male must follow the steps in the central sequence. In the first case, the male, instead of courting the female, landed next to her on the site. In the second, he attempted to copulate with her while she was still on the site. And in the third he courted her once she had flown up from the site.

In 18 of the 197 interactions in which the female did land on the site, the male simply left her to oviposit there. These will be discussed later. On another 25 occasions, the interaction was disrupted by another male interfering. This usually led to the female decamping from both males.

Finally, in 84 interactions, after landing on the site, making oviposition movements on it and being courted by the male, the female decamped. The male would initially chase her, but

eventually lose her or stop pursuit. Since only 66 females flew up slowly and landed, apparently accepting copulation, more females apparently rejected copulation than accepted. The reasons for this will be considered later.

Type II interactions are similarly presented in Fig. 25. Most of these interactions involved an intruder courting a female on another male's site, either while the resident was unsighted,

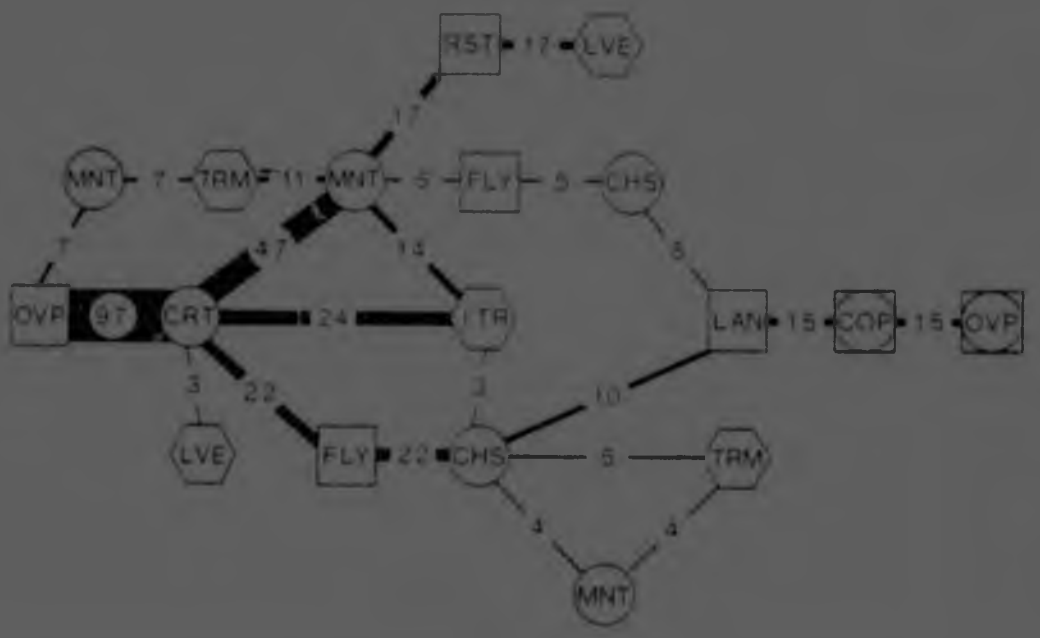

Figure 25. Flow-chart of type II interactions. See Fig. 24 legend for key.

or during or in between fighting. Some were the result of a new male taking over the territory, and very rarely a resident would court his own females. Many interactions (41 out of 104), of course, were interrupted, usually by the resident. On being courted, females appeared reluctant to fly up from the site. In most cases, perhaps because the males were usually involved in territorial interactions, and so appeared more aggressive than

those in encounter types I and III, the male ignored the female's wing-spreading display, and attempted to mount her on the site. Usually the female decamped, or resisted and shrugged the male off, whereupon he left her. Such attempts at mounting were far less successful (5 out of 47; 11%) than if the male waited for the female to fly up from the site (10 out of 22; 45%). On seven occasions the male mounted the female directly, without any preliminary courtship. These were particularly violent interactions, almost attacks, from which the female always decamped. All seven involved males which had just taken over the territory. The three instances where the males stopped courting involved residents which suddenly started courting their own females, and left when they gave the wing-spreading display.



Figure 26. Flow-chart of type III interactions. See Fig. 24 legend for key.

Type III interactions which involved a male finding a perched female are presented in Fig. 26. Here most males, on courting the female and receiving a wing-spreading display, simply left her. The rest of the interactions were terminated by the female either decamping, or attacking the male, who would then leave her.

6.3e Oviposition Site Selection

In type I interactions, females who landed and made oviposition movements on the male's oviposition site, appeared either to accept copulation or decamp. I hypothesized that the females were examining the sites for their suitability for oviposition, and so expected that some characteristic of the sites would be correlated with their rejection or acceptance. The sites were initially classified into four classes using two obvious characters, i.e. live (tree roots) or dead (driftwood), and with or without bark. Table 27 presents an analysis of the female's apparent acceptance or rejection of copulation, and therefore the oviposition site, with respect to the type of site tested. The distribution is clearly

Table 27. The numbers of females accepting or rejecting copulation after examination of the male's oviposition sites in type I interactions, relative to the type of site tested. The totals are lower than those in Fig. 24 because initially, oviposition site type was not recorded. Oviposition site types are discussed in the text.

Oviposition site	Acceptance	Rejection
Dead without bark	31	12
Live without bark	9	12
Live with bark	10	30
Dead with bark	6	24

non-random ($\chi^2 = 26.6$; $p < 0.001$), the females preferring barkless

driftwood while usually rejecting barked sites. The distinction however is not as clear as was expected. Towards the end of the study it was noticed that the barkless deadwood sites which were rejected were hard wood. Using a needle, numerous sites of each type were subjectively examined for softness. Dead and live sites without bark, having been in the water for some time, usually had a very soft outer layer. Sites with bark (especially when dead and thin) had a rather tough outer layer. Since the eggs are laid only in the outer 1.5 mm of the site, it seems possible that the females simply choose soft sites for ease of oviposition. It is clear, anyway, that they do have some preference.

It may appear strange then that on 36 occasions the female did not test the site in type I interactions (Fig. 24), and then accepted copulation. On 28 of these occasions, however, there was already one or more females ovipositing on the site. On only seven other occasions did a female test a site on which there was already another female ovipositing. Perhaps females "accept the judgements" of others in preference spending time examining the sites themselves. On the remaining eight occasions, when the site was unoccupied, two definitely involved females who had left the site and on returning were courted by a new male (either intruder or new resident). Since it was difficult to establish whether a female was returning, this may well explain the remaining six observations. Similarly, females who mated in type II interactions had already tested and chosen the site.

6.3 Results - Platycypha fitzsimonsi

This species is similar to P. caligata in morphology and colouration, except that the males' tibiae are not as expanded,

the tibial fronts are cream-coloured rather than bright white, and the abdomen dorsum is bicoloured with segments 1-4 red, 5 and 6 black, and 7-10 blue.

At both Oribi Gorge and R.N.N.P. the males defended territories around potential oviposition sites (sedge stems and driftwood at Oribi Gorge and driftwood at R.N.N.P.). Territorial interactions were not always as complex as in P. caligata (they often only involved chases), but all the behaviours described for P. caligata were observed at some stage, and males inspected oviposition sites by treading on them.

In the sympatric situation at Oribi Gorge the males of the two species were completely interspecifically territorial and defended similar oviposition sites. Indeed males were repeatedly observed to take over the territory of a non-conspecific and subsequently to use the same oviposition site. Interspecific territorial interactions did not appear as well structured as intraspecific interactions and usually simply involved vigorous chases with continuous display of the tibial fronts.

The mating behaviour of P. fitzsimonsi is generally similar to that of P. caligata. Males intercepted passing females and attracted them to their oviposition sites by displaying the dorsum of their abdomens. This differed from P. caligata in that only the last four segments, i.e. the blue part of the abdomen, were displayed by bending these down separately as a unit and waggling them from side to side. Once the female had landed on the site and was examining it by making oviposition movements on it, the male courted in a similar fashion to P. caligata males by displaying

the cream fronts of the tibiae. This courtship display differed from that of P. caligata in that it was less vivid because of the less expanded and cream-coloured tibiae, and also in that the tibiae were not vibrated separately but held together and swung vigorously from side to side. Receptive females flew up, allowed the male to copulate, and were then returned to the oviposition site in tandem by the male. Copulation duration was not measured but seemed similar to that of P. caligata, i.e. about 60 s. Twenty-two successful and sixteen unsuccessful such interactions were observed at Oribi Gorge. All other interactions and courtships, i.e. not involving interception and attraction of the female and her examination of the site, were unsuccessful.

In the sympatric situation at Oribi Gorge males reacted to, intercepted, and attempted to attract any passing female. Their coexistence must therefore depend on differential mate recognition by the females of the two species. A brief attempt was made to establish whether this involved the differences in the males' attraction and courtship displays noted above. A major difficulty was that the cryptically coloured females of the two species were also indistinguishable to me. So females were caught in copula, marked, and subsequently considered to be of the species of the male with which they were caught. Observations of subsequent interactions by such females yielded the following results. Six P. fitzsimonsi females were subsequently successfully attracted by P. fitzsimonsi males and none by P. caligata males. All six mated. Four P. caligata females were attracted by P. caligata males (and one mated) and one by a P. fitzsimonsi male. Also, two unmarked females were observed being attracted by males of both species in succession. It appears that females respond

preferentially but not exclusively to the attraction displays of conspecific males. The three interspecific attractions observed led to the female decamping when the male started courting. Although hybrid males were specifically searched for, none were found. Assuming such hybrids would be viable, their absence, and the above observations, suggest that interspecific attractions do not lead to copulation, probably because the courtship displays are different.

The importance of this difference in courtship displays was partially examined in another brief experiment conducted with the allopatric population at R.N.N.P. Some males' meso- and metathoracic tibiae were painted bright white to simulate those of P. caligata males (the prothoracic tibiae were too difficult to paint). Of 17 successful attractions of females by these males, 13 led to copulation. Clearly this change did not prevent females from recognising these males as conspecific. Perhaps the crucial difference between the two courtship displays is the difference in patterns of tibial vibration.

6.3 Results - Chlorocypha consueta

The males of this species have unexpanded tibiae which are white anteriorly, and the abdomen dorsum is uniform pink-red. Otherwise they are morphologically similar to the Platycypha species and the females are again very similar. The stream on which they were observed was similar to those where the Platycypha species are found in South Africa, i.e. swift-flowing and rocky with little emergent vegetation, and driftwood or tree-roots for oviposition sites.

The behaviour of this species was generally similar to that of the Platycypha species but differed in the displays. The males

were territorial around the oviposition sites which they examined as usual. However the territorial interactions were completely unstructured, involved no tibial displays, and simply consisted of chases and jittery side-by-side and circular flights. Occasionally one male would fly in front of the other with his abdomen lowered, thus displaying the pink-red dorsum.



Figure 27. The courtship display of Chlorocypha consueta. As reconstructed here using a pinned specimen, the male arches his abdomen above his head. The white fronts of the unexpanded tibiae can be seen.

Males intercepted passing females and attracted them by displaying the entire pink-red abdomen as in P. caligata. The females examined the sites as usual while the males courted. Again the courtship display was different. The male displayed

the white fronts of his tibiae by holding them together, without vibration or swinging, below the thorax. This created a small patch of white colour which was far less vivid than the Platycypha displays. At the same time the male arched his abdomen above his head in a dramatic display of the pink-red dorsum (Fig. 27). As in the Platycypha species successful courtships led to copulation away from the oviposition site after which the male returned the female to oviposit. Five measured copulations had durations of 101 ± 33 s. Of 18 such interactions observed, six led to mating. All other interactions away from oviposition sites were unsuccessful.

6.3 Results - Rhinocypha unimaculata

The males of this species have unexpanded tibiae, but the fronts of both the tibiae and the femora are white. The abdomen dorsum is black out the distal two thirds of the hind wings are darkly pigmented. Approximately half-way along the hind wings there is a transverse hyaline band or window in this pigmented section, which reflects a brilliant green iridescence. Again the females are cryptically brown-coloured.

The following is a description of a single courtship sequence filmed by Dr. J.M. van Brink. The female was making oviposition movements on a piece of driftwood at the water surface and wing-spreading while the male courted her. The hovering male described an arc around and facing her while continuously displaying the white fronts of his legs in a similar fashion to C. consueta, i.e. held stationary together, creating a clear white patch below the head. He repeatedly held the hind wings stationary, forward and downward for brief periods during the courtship while continuing to hover with the fore wings. This created a brilliant

flashing display of the green bands on the hind wings. The pair apparently copulated away from the site and the male returned the female to oviposit on it.

6.4 Discussion

6.4a Platycypha caligata

These chlorocyphids, particularly Platycypha caligata, exhibit complex territorial behaviour, and dramatic and varied male courtship displays. Because the data collected on P. caligata are the most detailed, the evolution of this mating behaviour will be considered using this data first. Then support and additional conclusions from the work on the other species will be discussed.

This mating behaviour centres around the oviposition sites which the males defend, and which the females apparently test for suitability for oviposition, possibly on the basis of their softness, during the courtship. The observations reported here show that females mate almost exclusively with territorial males which defend suitable oviposition sites. The few non-territorial males which mated were among those that courted already ovipositing females (who had already chosen the site). Although the possibility was not examined in detail, casual observations suggested that the females do not choose males themselves on any obvious basis. For example, they were sometimes observed accepting copulation with physically tired or old males who struggled to lift them while attempting copulation. Four females on seven occasions attempted to mate with a male who, due to an abdominal deformity, was unable to achieve the copula position.

Similar mating systems involving female choice of oviposition sites defended by males (Jacobs 1955; Pajunen 1966; Waage 1973), or at least male defence of areas where females are more likely

to appear and oviposit, have been described for a number of odonate species (Campanella and Wolf 1974; Campanella 1975; Pezalla 1979).

In none of these studies were non-territorial males so consistently rejected by the females, nor the females' examination and choice of the oviposition sites so clearly a central part of the mating system as in P. caliqata. In the study most similar to that presented here, Waage (1973), using the same three pair-formation categories used here, found that in Calopteryx maculata, females were statistically equally likely to mate in the three interaction types. In P. caliqata. females never mated in type III interactions (0 out of 108), occasionally in type II interactions (15 out of 104), and mostly in the type I interactions (91 out of 352) (χ^2 = 37.7; $p < 0.001$). However, Waage (pers. comm.) has recently repeated his work on another population of C. maculata and found more consistent rejection of non-territorial males. As will be seen later, these later results fit the theory presented here better, because C. maculata and other calopterygid species (e.g. Heymer 1973; Waage 1973) also have elaborate courtship displays involving attraction of females to oviposition sites and their examination of them.

Following ideas developed by various authors (e.g. Emlen and Oring 1977), I speculate that the evolution of this mating behaviour resulted from the nature and distribution of the oviposition sites. In most Zygoptera and Anisoptera oviposition is either endophytic in emergent vegetation, or exophytic wherever there is suitable water (Bick 1972; Corbet 1980). As a rule

neither type of site is readily economically defendable (sensu Brown 1964). The aggressive or territorial behaviour exhibited by the males of these species seems to involve defence of an area for interception of females, and sometimes reduction of interference in mating and sometimes of oviposition (Johnson 1964). Choice of oviposition site is almost entirely dependent on the female, and mostly, at least microgeographically, subsequent to mating (e.g. Bick 1972).

As soon as a new species starts ovipositing on a site which is clumped and patchily distributed in discreet units, the males would centre their defence on these sites, perhaps initially because of increased female activity at these areas. Males not defending sites would be at a disadvantage simply because they would not intercept as many females (Campanella and Wolf 1974; Campanella 1975; Pezalla 1979).

Species of Calopteryx use clumps of waterweeds, which are moderately discreet sites (Pajunen 1966; Heymer 1973; Waage 1973). Waage (pers. comm.) has observed Calopteryx maculata using driftwood oviposition sites in mountain streams. Again, if it is assumed that calopterygids evolved in such habitats (perhaps as pleiostocene refuges) and have subsequently enlarged their ranges, this would better fit the present theory. P. caligata oviposition sites are exceedingly discreet, individual pieces of driftwood or roots, among which there are usually few, at least relative to male density, suitable sites. Under such conditions, females would be selected to mate only with territorial males, and to make some examination of the site prior to mating, since females unselectively mating with

non-territorial males or males with unsuitable sites would have to remate, often repeatedly, in order to oviposit on a suitable site. In this study, females were seldom observed ovipositing alone (19 observations), and this was usually on hidden, often unsuitable sites for only short periods.

In this scenario, a male should mate with a female before allowing her to oviposit on his site. Yet on 18 occasions (Fig. 24) the male attracted the female to his site and then simply left her to oviposit there, without mating with her. Similar behaviour has also been reported and discussed for Calopteryx maculata by Waage (1979c) and Alcock (1979). As noted by Alcock (1979) most of these appeared to be instances where ovipositing females left the site and were now returning. Having found a good site, females would be expected to return to it if disturbed and be uninterested in remating, and females were observed to be persistent in returning to a chosen site. Similarly, assuming P. caligata males use their penes to remove sperm from previous matings from female seminal receptacles (as Waage (1979b) demonstrated for C. maculata males (see also Miller and Miller 1981; Waage 1982)), males should attempt to prolong a female's period of oviposition on their sites, since they are only assured of paternity for this period. Males did repeatedly attempt, often successfully, to re-attract restless females which flitted about, or were leaving

the site. Although these observations and those of Alcock (1979) suggest that males can often distinguish returning females from new ones, how they might do so is unclear (Alcock 1979), and in fact Waage (1979c) concluded that they do not. Males did sometimes leave new females, and furthermore, females which landed on a male's site with already ovipositing females, while he was interacting with another male or otherwise distracted or unsighted, were ignored. Such confusion may have been the cause of the four unsuccessful interactions in Fig. 24 where the male did not court the female, and also the five where the male eventually attempted to mount the female while she was still on the site. While Waage (1979c) suggested that such confusion results from males compromising their changes of mating with a new female for the certainty of guarding a mated female, Alcock (1979) argued for the reverse. Observations of new females being intercepted before the mated ones return may clarify this problem. Where there was no possibility of confusion, however, the males acted appropriately. Thus a female landing unnoticed on male's empty site was always courted when discovered. Similarly, when an intruder took over a territory with ovipositing females, he would always persistently court and attempt to mount them, or even drive them from the site, hence the 7 interactions in Fig. 24 where the male, without preliminary courtship violently mounted, perhaps attacked, the ovipositing female.

Strangely perhaps, P. caligata males do not show the separate guarding behaviour of their females that many other species, including Calopteryx spp. exhibit (Alcock 1979; Waage 1979c, 1982; Corbet 1980). However, the highly developed normal territorial behaviour of this species may be sufficient. On the other hand, P. caligata males keep the females in tandem after copulation and return them to their sites, whereas C. maculata males release them and they have to find the site themselves, and are sometimes intercepted first by another male.

Once females mate almost exclusively with males with suitable sites, males would be selected for the ability to recognise such sites. In fact, their visual recognition of sites appeared to be better than that of females. Thus, whereas females were observed attempting to oviposit on rocks on 18 occasions (once ovipositing at a site females often tried nearby objects as well), males were only observed treading on rocks on 2 occasions. The treading behaviour of males seems to be a further examination of the site, but the actual method of assessment is unclear. Jacobs (1955) reported that males of the dragonfly Platnemis lydia (exophytic ovipositors) simulated oviposition while examining sites they later defended. Clearly P. caligata males could not examine sites as the females do, and the treading behaviour may be the best alternative.

Again, once females insist on examining the males' sites before mating, males would be selected to co-operate in attracting a female (a behaviour facilitated by the blue abdomen), and then to leave her on the site and respect her wing-spreading display

until she flies, lands and indicates acceptance of copulation by not wing-spreading. Perhaps the actual courtship display evolved in this context. The male would have repeatedly attempted to mount the female on the site and have been repeatedly repulsed by her wing-spreading display, resulting in an equilibrium in which he hovered, facing her with his legs extended. Such a behaviour could readily be ritualised into a display with additional elaboration of tibial morphology and colouration. Similarly one may postulate that the expanded tibiae and the white colouration evolved initially in the context of male-male territorial interactions. Extension of the legs is a preliminary to attack, and such an intention movement could have been elaborated. It is however difficult to develop a similar scenario for the evolution of the red colouration of the backs of the tibiae.

6.4b The Other Species

The other three species were studied to test and elaborate some of these ideas. Platycypha fitzsimonsi and Chlorocypha consueta clearly use similar oviposition sites and have similar mating behaviour to P. caligata, involving male attraction of females to the defended oviposition sites which are examined while the male courts, and the females again do not mate with non-territorial males. Rhinocypha unimaculata appears to be similar too, but needs more study. All the species have courtship displays, which fits the theory presented here. These species represent three major genera in the Chlorocyphidae (there are two other major genera and five smaller genera) (Davies 1981). It is therefore expected that courtship displays, of perhaps even other forms, will be found in all Chlorocyphids, especially

since all African Chlorocypha and Platycypha species have at least coloured abdomens and often coloured tibiae (Pinhey 1966) (see also Paulson 1981).

A test of the generality of the theory presented here would be to search for species with male courtship displays, and in which, however, the males do not defend territories around oviposition sites which the females do not examine before mating. To date I know of no such detailed examples (but see Williams 1977), but a fascinating test situation is available. In the zygopteran family, Platycnemididae, Platycnemis pennipes in Europe have slightly expanded tibiae with white fronts (e.g. Bucholtz 1956), two Copera species in Malaysia have moderately expanded and coloured meso- and metathoracic tibiae (Furtado 1974), and Platycnemis foliacea in Japan has extremely broad white tibiae (e.g. Asahina and Eda 1956 ; Eda 1958). Unfortunately the mating of these species has never been fully observed and courtship has never been seen. I predict that there are no courtship displays in these species because the males do not defend territories around oviposition sites, and oviposition is in tandem outside what territories there may be (Bucholtz 1956; Furtado 1974).

Bucholtz 1956) and Furtado (1974) report that these platycnemidid males use the tibial fronts in flash displays in territorial interactions, as the Platycypha males described here do. If they do not have courtship displays this tibial elaboration must have evolved in a territorial context.

In the chlorocyphid species, the finding that C. consueta males only use their coloured tibiae in the courtship display suggests that this colouration evolved in this context. On the

other hand, that the Platycypha species only use the red posterior surfaces of their tibiae in territorial interactions demonstrates that at least this colouration evolved in that context. Thus tibial expansion and colouration in chlorocyphids may have evolved in either context. Clearly the different courtship displays of C. consueta and R. unimaculata evolved independently of territorial contexts (Paulson (1981) confirms that Rhinocypha species do not use the wing display or the tibiae in male-male interactions) and the discovery of still other courtship displays may be expected. The display of only the tip of the abdomen as a separately articulated unit has been observed here in P. fitzsimonsi and also in Chlorocypha glauca (Neville 1960; R.M. Gambles pers. comm.). It is probably used by all species with bicoloured abdomens as an attraction display, and perhaps, as a courtship display (Neville 1960). The display of the hind wings in R. unimaculata is similar to the various courtship displays described for Calopteryx species (e.g. Waage 1973). This is probably a convergence and suggests that many other species in other zygopteran families with coloured or iridescent wings may also have courtship displays involving these.

Specific-mate recognition in the present species clearly involves an initial visual recognition of the female by the male, which usually seems to require the female to be flying. Males seldom responded to stationary females (except those ovipositing) and would often perch next to them. This is followed by female recognition of the males' attraction and courtship displays. Differences in female colouration and male recognition of females is unlikely to be sufficient to facilitate species coexistence. Such sympatric coexistence in chlorocyphid species probably depends entirely on differences in male attraction and/or

courtship displays, and female specificity in recognition of these, as appears to be the case for P. fitzsimonsi and P. caligata described here. Chlorocyphid male anal appendages are not used in taxonomy because they vary little between species, so specific female tactile recognition of these (Chapter 5), if present, is probably also not important in sympatric coexistence.

Finally, the behaviour of these species of ovipositing on driftwood and treeroots (which is atypical of Zygoptera, and which are the discreet and scarce sites invoked as the initial component of the theory developed here), seems to be related to their usual habitat (at least in Southern Africa), i.e. swift-flowing, rocky mountain streams. There is no emergent vegetation, so these are the only available sites. Interestingly, the only other group of Zygoptera regularly and abundantly found on such streams, the Chlorolestidae, appear to oviposit in tandem on twigs and branches above the water (personal observations), which is another solution to the lack of endophytic oviposition sites. The occasional use of emergent vegetation by the Platycypha species at Oribi Gorge may, as was suggested earlier for the Calopteryx species, be a secondary development, as is the recent colonization of Lake Malawi by P. caligata (M. Picker pers. comm.; Pinhey 1981).

Chapter 7. GENERAL DISCUSSION

I will attempt to fit these studies into an overall discussion of mating behaviour and its evolution. Firstly, the general structure of Specific-Mate Recognition Systems (SMRS's) will be considered, and how odonate and Drosophila courtship fits into this framework will be described. Secondly, the relevance of this work to the theory of sexual selection will be discussed because sexual selection is usually considered to be important in the evolution of mating behaviour and especially courtship displays. Finally, how SMRS's change and evolve in allopatry will be considered.

7.1 Specific-Mate Recognition Systems

7.1a General

The SMRS comprises the communication system which leads up to syngamy in sexual organisms. It therefore includes what is often separated as sex and species recognition, and is similar to what the ethologists defined as courtship. Thus Morris's (1956) definition of courtship as "...the heterosexual communication system leading up to the consummatory sexual act" is appropriate. Paterson (1978, 1980) considers a schematic SMRS to consist ideally of a chain of stimulus-response pairs, where proceeding to the next step in the chain is conditional on the success of the previous step. Such courtships have been described by various ethologists for many animals including fish, butterflies and flies (e.g. Morris 1956; Bastock 1967). Perhaps the clearest and neatest demonstration of such a system is that of Stich (1963) in a tipulid fly. As Morris (1956) noted from his work on stickleback courtship, although a schematic chain of stimulus-response pairs can be drawn up, not

every successful courtship follows this exact sequence. For example, many (probably depending on context) miss out one or more steps in the chain. This must be a general feature because the sexes may meet in different situations and contexts. This is especially so in organisms that do not use spatially accurate external cues or long distance signals to coordinate meeting. One of the most extreme examples of this is Jackson's (1977) salticid spider, Phidippus johnsoni, which uses a more visually based courtship when a male encounters a female outside her nest, but a more tactile and chemically based courtship if he finds her inside the nest. The chlorocyphid damselflies described in Chapter 6 are similar in that, when a male discovers an ovipositing female, the attraction display using the lowered abdomen is of course dispensed with, yet some females mate in these interactions. Other omissions or differences in the courtship are not, however, acceptable and lead to failure (6.3d).

7.1b Odonata

In general, odonate mating benaviour fits the schema of an SMRS rather well, as described in Chapter 5. Two further points can be made. Firstly, individual steps need not be species specific. For example, Enallagma and Platycypha males will respond to other congeneric females. Furthermore, Paulson (1974) and Tennessen (1975) report that Enallagma spp. males clasping non-conspecific females are not immediately aware of the fact, and only eventually release the struggling females.

Secondly, the tactile recognition system illustrates what is meant by a stimulus-response pair rather well. The male's anal appendage (the signal or stimulus) complementarily fits into the

female's groove to stimulate all the sensilla. In other sensory modalities and other animals, this complementary matching may similarly involve largely peripheral filtering (i.e. at the sense organs), as in the female frog's ear (e.g. Capranica et al. 1973; Walkowiak 1980) or the male moth's antenna (Seabrook 1978), or central processing (i.e. in the C.N.S.) as appears to be the case with bird song (Marler 1976). That is, the receptor system is peripherally and/or centrally "tuned" to receive the stimulus, which constitutes recognition.

7.1c Drosophila

Drosophila courtship has fascinated many workers over the years and literally hundreds of papers have been published on the description and experimental analysis of the courtship of various species. Nevertheless, we do not yet have a detailed understanding of the courtship of D. melanogaster. I will first review our current understanding of the stimuli involved in this complex interaction, and then consider how it fits the SMRS schema.

Spieth (1974), in the only brief report on behaviour in the wild, concludes that, both sexes having arrived at fermenting fruit at the same time, the males respond visually to, and approach and tap any fly of the appropriate size. Movement seems to facilitate this initial reaction. In my opinion (see Appendix A) the evidence for the involvement of volatile chemicals in the stimulation of tapping or subsequent courtship by the male is not strong, but like the visual stimuli they may be used but are not crucial. Thus in the dark, males will tap a fly they bump into.

In Appendix A I provide additional evidence that the contact chemicals presumably gained by tapping are very important. After

tapping, the male loses interest in other males and, usually, females of other species, and sometimes even fertilized conspecific females (Connolly and Cook 1973; Spieth 1968, 1974). Hall and his colleagues have recently isolated a 27 carbon chain chemical from females (but not males) which stimulates male courtship (pers. comm.). This would be only barely volatile, and its limited volatility may explain the contradictory results reported in the literature, and which I attempt to resolve in Appendix A. That is, in real courtship situations it may only be perceived by actual contact, but in some close range experiments its limited volatility may allow stimulation of courtship.

The male then courts by extending and vibrating one wing at a time, licks the female's genitalia and attempts copulation. The stimuli transmitted by the male's wing vibration have been the subject of some debate (e.g. Bennet-Clark et al. 1976; Averhoff and Richardson 1976a; Averhoff et al. 1979). There has been considerable work on the sounds generated by this vibration. The patterning of the sound varies between species (see Chapter 4), and even intraspecifically (using taxonomic species) (e.g. Miller et al. 1975; Chang and Miller 1978; Ikeda et al. 1980). Schilcher (1976b) has confirmed Bennet-Clark and Ewing's (1969) demonstration that artificial auditory stimuli of the correct patterning increase the mating success of wingless males. Other authors have emphasised the importance of volatile chemical stimuli (see Averhoff et al. 1979), which may be transmitted in an air-stream generated by the male's wing vibration. The best evidence for this is from Averhoff and Richardson's (1976b) work on lack of mating in inbred strains, and the work of Ehrman and others on the phenomenon of rare-male mating

advantage in Drosophila (e.g. Ehrman 1969; Leonard et al. 1974; Leonard and Ehrman 1976). The reality and interpretation of both phenomena has been questioned (e.g. Bryant 1979; Bryant et al. 1980; Kence 1981; Markow 1980), but the volume of data supporting the latter phenomenon suggests that at least it is not completely artifactual. Recently Spiess and Kruckeberg (1980) have reanalysed the problem of the causation of the rare-male effect and conclude that it simply involves avoidance by the female of the first male phenotype encountered, independent of the actual proportions of males present. This may still be olfactorily based.

The other line of evidence on this question involves removal of the female's aristae and antennae, the idea being that removal of the aristae only removes perception of auditory stimuli (by removing the movement which stimulates the Johnson's organ on the antennae). Removal of the entire antennal complex would then also remove any olfactory stimuli. Petit (1958) (see Manning 1967b) found no difference between the effect of these two operations on the female's mating propensity, suggesting that olfactory stimuli are not important. However, her experiments were 48 h long, which would perhaps have allowed the antennaeless females to mate eventually. Petit and Nouad's (1975) methods have the same problem. I gained similar results in 48 h tests (Table 21). As Manning (1967b) pointed out, much shorter time periods must be used. For example, in Table 11 it can be seen that in 2 h tests antennaeless females are unlikely to mate, whereas most mated in 48 h tests. Manning (1967b) nevertheless confirmed Petit's results in 30 min single pair tests. My results disagree with this (Table 11), i.e. aristaeless females were far more likely to mate than were antennaeless females.

A major problem here is that removal of the antennae is a far more traumatic operation than removal of the aristae, which may explain the difference in mating propensity. Also, Averhoff et al. (1979) have presented evidence that the aristae may also perceive chemical stimuli. Ewing (1978) has nevertheless confirmed that the antennal complex of at least one Drosophila species can receive auditory stimuli. It seems then that both types of stimuli are transmitted by the male's wing vibration. A possibility worth investigating is that the form of vibration producing pulse song is transmitting auditory stimuli, while that producing sine song is actually transmitting the olfactory stimuli.

Before proceeding, yet another problem, that of strain differences, can be mentioned. D. melanogaster is an experimental animal of long standing and different strains have undoubtedly acquired slightly different mating behaviours. An extreme example is the report by Burnet and Connolly (1974) that males of one strain court without first tapping the female. Similarly, the importance of the male's wing vibration may vary between strains (Appendix B).

After wing vibration the male licks the female's genitalia, grasps her abdomen with his sexcombs and attempts copulation. There can be no doubt that the licking, like the grasping (Cook 1977), is important in preparing for attempted copulation, for example, in locating the female's genitalia for the thrust of his genitalia. It has also long been suspected that tactile and/or chemical stimuli pass in either direction between the male and female during this behaviour. This may well be so, but the finding that the proboscisless males are less handicapped than are sexcombless (and foretarsiless) males (compare Tables 15 and 16) shows that these

stimuli are less important than the purely mechanical grasping action of the sexcombs.

It has been argued by some authors (e.g. Manning 1965) that the courtship of D. melanogaster exemplifies an alternative form of courtship to the chain of stimulus-response pairs in that, having started courting, the male will go through the entire courtship without any response from the female. This is based on observations that males will court and even copulate with etherized (Streisinger 1948) or decapitated females (Spieth 1966; Cook 1975; Chapters 3 and 4). Two points are important here. Firstly, in attempting to repeat Streisinger's experiment I had to include normal females, which the males courted enthusiastically, before any would court the etherized females. Males are similarly reluctant to court decapitated females unless normal females are present (see also Spieth 1966). Furthermore, to get copulation with the etherized females it is necessary to squeeze their abdomens before placing them with the males, perhaps because the vaginal plates have to be opened. Thus, while it is quite true that the males will fully court these females, they usually need to be already excited to do so.

Secondly, because of our lack of understanding of the stimuli involved in this courtship, especially those exchanged during licking, we do not know if there is some sort of female "acceptance" signal, perhaps perceived by the male during licking, which in normal courtship releases attempts at copulation. Some authors have pointed out that lack of, or at least reduced, female movement facilitates copulation (see Cook 1979, 1980; Hall et al. 1980; Chapter 2). Not decamping may be a form of "encouragement" if not an acceptance signal. Others have reported that the female opens her vaginal

plates (e.g. Spieth 1974). This is very difficult to observe, but may be important. At one stage I thought that the yellow males were not perceiving this "acceptance" signal and so did not complete attempts at copulation, but as reported in Chapter 3, this cannot be confirmed. Nevertheless, these mutants may yet illuminate this problem.

Examination of other species has shown that when the flies use our major sensory modality, vision, then we observe acceptance displays. For example, D. subobscura (Rendel 1945; Pinsker and Doschek 1980) and D. auraria (Grossfield 1968), both completely light dependent in their courtship , have acceptance displays involving wing opening by the female. Many other species also have such displays (Spieth 1952; Ikeda et al. 1981). To us therefore, these courtships fit a reaction chain better, so it is still probable that light independent species such as D. melanogaster also have a similar acceptance signal involving chemical or tactile stimuli, even if this is overridden by, or unnecessary for, excited males. So even D. melanogaster courtship may yet be shown to involve a stimulus-response series, if not a rigid chain of fixed order.

Interlinked with this are the concepts of heterogeneous stimulus summation and more particularly female courtship summation. These hold that the female simply summates the various stimuli directed at her by the male until her mating threshold is reached. Support for this, other than that questioned in Chapter 2, is found in the observation that removal of any of the stimuli (by removal of light or wings or proboscis, etc.) does not completely prevent copulation. This has been noted in other animals too (e.g. Colwell and Shorey

1976; Bell 1980). In Chapter 2 I argue that progressive male wing removal affects mating propensity by decreasing the female's ability to perceive the signal, rather than by reducing "sexual stimulation" of the female. This point is well illustrated by Grossfield's (1968) results on D. auraria. The female's wing opening acceptance display in this species can hardly be considered to be summated by the male. Rather it simply evokes an immediate mounting response by the already courting male. Yet partial progressive removal of the female's wings causes a progressive reduction in mating. This can only be due to progressive reduction of perception and recognition by the male. In this case total removal of the wings totally prevents mating. In D. melanogaster total removal of the male's wings does not totally prevent mating, presumably because the female can still recognise the male as a mate on the basis of other stimuli. Her reluctance to mate is not due to a reduced rate of heterogeneous summation, but due to her difficulty in recognising the male. That is, there is some redundancy in the SMRS. The same applies when the males use visual and contact chemical stimuli in recognising the females (e.g. D. mauritiana) but will sometimes court in the absence of either. This is comparable to Morris's (1956) finding that steps can be left out and still lead to mating.

Thus Drosophila courtship does fit the SMRS schema with the addition that individual steps can involve more than one stimulus. It might be best to view a generalized SMRS as consisting of a series of reciprocal stimulus-response pairs not necessarily organised in a rigid chain.

7.2 Sexual Selection

Darwin (1871) argued that what he called sexual selection has

been responsible for the evolution of most secondary sexual characters, which would include those used in specific-mate recognition Darwin effectively defined sexual selection as involving any differential reproductive success, while natural selection was relegated to differential survival. Thus he says (Chapter 21, p 939): "Sexual selection depends on the success of certain individuals over others of the same sex, in relation to the propagation of the species; whilst natural selection depends on the success of both sexes, at all ages, in relation to the general conditions of life." A number of authors have questioned this general usage of the term sexual selection (e.g. Huxley 1938; Bastock 1967). Recently sexual selection theory has enjoyed considerable popularity, especially in the light of refinements such as those of Emlen and Oring (1977). It is however invoked in a wide variety of way varying from Darwin's broad usage to restriction to the so-called run-away or positive feedback selection process proposed by Fisher (1930) and elaborated by O'Donald (1962, 1977). Although the work reported here was not undertaken to investigate any of these ideas, some of it is relevant, so this will be discussed briefly. A general overview of sexual selection theory, as now understood, can be obtained from Blum and Blum (1979). The most controversial and interesting aspect is the proposed selection or choice by females of "fitter" males. The term "choice" need not imply concious judgement in our sense, but only female recognition of appropriate habitat and/or male. Theories and models assuming this abound, but there is little empirical evidence for it. It is this aspect I will discuss.

The evolution of the mating behaviour of the chlorocyphi

damselflies described in Chapter 6 would be explained by most authors in terms of sexual selection involving female choice. However it should be noted that at no point in the explanation offered in Chapter 6 was it necessary to invoke female choice of fitter males. Thus, for example, the evolution of territoriality need only involve the selection of males which defend oviposition sites. The females choose the oviposition sites, the males coming incidentally with the sites. This point is becoming appreciated in the study of territoriality in birds. Thus Searcy (1979) and Yasukawa (1981) attempt to separate female choice of territories and males in blackbirds and conclude that territory quality is the major variable determining where females breed. Even then the situation remains very complex (Lenington 1980). Female choice of some sort of resource defended by males is of course well documented in many animals (e.g. Emlen and Oring 1977). Jones (1981) presents a particularly good experimental analysis of this in a fish. Although many authors argue that the females are actually choosing fitter males on the basis of their ability to defend good territories, this is unnecessary to explain the evolution of such mating systems.

Demonstrating some form of female choice of particular males independently of territory quality or any other factor, such as food in Thornhill's (1979) scorpionflies, remains a major problem. The nearest so far is the demonstration of female choice of larger males in toads (see Gatz 1981) and sculpins (Brower and Brown 1980). Even then a number of problems remain with accepting that this leads to evolution of any kind. These are best illustrated by the, to my knowledge, first long term study of reproductive fitness in this context. McGregor et al. (1981) report that in their great tits,

firstly, life-time reproductive success after three generations is normally distributed with song repertoire size, and secondly, repertoire size is not heritable.

When considering the evolution of the attraction displays of the chlorocyphid damselflies it is similarly unnecessary to invoke female choice of fitter males. Calopterygid damselflies also attract females to oviposition sites, some without coloured abdomens (e.g. Waage 1973), as may the males of the few chlorocyphid species which do not have coloured abdomens. The evolution of the colour need only involve improved efficiency of the ability of the female to follow the male, perhaps into particularly dark areas such as crevices where oviposition sites are lodged. Indeed most aspects of SMRS's probably evolve to improve efficiency of communication in particular habitats or circumstances.

Explanation of the evolution of the courtship displays is more difficult. For example, why does Platycypha caligata have such an elaborate version of the extension and display of the tibiae, involving expansion and colouration of the tibiae and their vibration. In my opinion there are too many unknowns in this example to suggest a reasonable explanation. For example, one would like to know whether males of species with unexpanded and uncoloured tibiae (see Pinhey 1966) also display them in courtship, and what the phylogeny of this group of species is. Nevertheless one interesting point can be made. This sort of elaboration would usually be explained in terms of Fisher's (1930) model. A problem seldom noted with this model (but see O'Donald 1977; Borgia 1979) is that his and O'Donald's (1962, 1977) subsequent mathematical treatments only involve spread through a population of a single increment in a male

trait and the female preference for this increment. In order to get a run-away process from this it is necessary that the female's preference be open-ended, i.e. females must, as an integral part of their mating responses, always respond more to supernormal stimuli. Otherwise one must always wait for a further small male trait change related to natural selection, and a female preference for it to arise.

None of the examples of supernormal stimuli routinely quoted in textbooks involve female mating responses. Unfortunately most work on female mating responses has involved removal of aspects of the courtship (e.g. Bateson 1948; and this thesis), which is expected to impair mating because the SMRS has been affected. For sexual selection, what is needed is demonstration that females prefer additions, not reject removals. The best work I am aware of is the demonstration by King and West (1977) and West et al. (1979) that cowbird females respond with copulatory postures to isolate male song more readily than to normal male song. The reason for this is not clear, especially since isolate song is more "simple" than normal song. Nevertheless, this and Searcy and Marler's (1981) similar work on female song sparrow responses to male song, are the first experimental analyses of bird song using female bioassays. The neatness and relative simplicity of their experimental systems should allow testing for open-ended female preferences for features such as song complexity or repertoire size. It might be remembered that in Chapter 6, in an attempt to examine the differences in the courtship displays of P. caligata and P. fitzsimonsi males, I painted the P. fitzimonsi males' tibiae bright white. In addition to partially simulating P. caligata males, this would constitute a

very relevant supernormal stimulus. Thirteen (76%) of the 17 successful attractions by these males led to copulation. This is a bit higher than in normal P. fitzsimonsi interactions at Oribi Gorge where 22 (61%) of 36 successful attractions led to copulation, and considerably higher than in P. caligata where 91 (34%) of 265 successful attractions led to copulation (Figure 24). However, because I was examining the SMRS differences, I chose four particularly good oviposition sites (waterlogged barkless driftwood) to place in my experimental area, so that possible rejection of the altered males by the females could not be ascribed to poor oviposition sites. The increased rate of acceptance may only be related to these good sites. A well controlled repetition of this experiment would be interesting.

Another problem with much of this work is that the immediate function of the particular steps in the SMRS is not always clear. For example, the function of the attraction display is clear, but that of the courtship display is not. One might speculate that it helps position the female on the oviposition site, because the male initially courts from behind the oviposition site as the female lands. In Drosophila this is even more difficult, but it may be suggested that some aspects evolved in the context of circling the female to prevent her from decamping. An interesting example of this functional approach is Obana's (1979) argument that the "mating dance" of male Bombyx mori moths in fact is necessary for location of the female. I have attempted a functional approach in explaining the evolution of the (for Odonata) complex mating behaviour of chlorocyphids and calopterygids in terms of male cooperation in female examinati[illegible] oviposition site. An

interesting point of comparison is that in the Anura, complex courtship has only evolved in those species, especially in the family Dendrobatidae, where the male has to lead the female to a prepared oviposition site (Wells 1977).

Finally, the work reported in Chapter 2, questioning the concept of female courtship summation in Drosophila, is highly relevant to theories of sexual selection in that sexual stimulation, mediated by female courtship summation and mating thresholds, is a general assumption in most models of intersexual selection in which the female is supposed to choose males on the basis of the intensity of their courtship. However, as Kennedy (1966) argued, such "female coyness" may not be real, because successful courtships in the wild may be relatively short. It must be noted, however, that there are some reports in some insects of prolonged courtships in the wild eventually leading to copulation (Alcock and Pyle 1979; Las 1980; Tricca and Trujillo-Cenoz 1980), so this theory may be relevant in some species.

7.3 SMRS Changes in Allopatry

7.3a General

We come now to the question of how SMRS's change in allopatry, because, as discussed in Chapter 1, this must be our general if not exclusive model of speciation. Although most workers accept speciation in allopatry as the major model, they are usually talking of species as reproductively isolated groups, which includes those only exhibiting post-mating isolation. Reinforcement of "premating isolating mechanisms" would then be necessary to achieve full speciation in the sense of species used here. Hence complete change of SMRS's (evolution of complete pre-mating isolation) in complete

allopatry is actually not generally considered. Even Mayr (1970) ends up fully accepting the reinforcement model when species are intersterile. It is only when considering interfertile species that he, and other authors (e.g. Carson 1978), attempt an explanation in complete allopatry. In Mayr's case, his commitment to the concept of isolating mechanisms led him to propose only one mode of change in allopatry, i.e. pleiotropic effects of other genetic changes during differentiation in allopatry. This was simply because he recognised that isolating mechanisms are ad hoc mechanisms which only function in sympatry and so can only evolve directly in this context. The alternative view supported here is that allopatric changes in SMRS's might result incidentally in pre-mating isolation in subsequent sympatry, an explanation applicable to both interfertile and intersterile species. An analogy for this argument was presented in Chapter 5, where the mechanical isolation observed in Enallagma damselflies was explained in terms of incidental effects of changes in a tactile recognition system.

Approaching this entire problem from the more positive view of Paterson's recognition concept, where the SMRS is considered to be an intraspecific adaptation to effect syngamy efficiently in the preferred habitat of the species, allows consideration of a number of possible causes of changes in allopatry in addition to pleiotropic effects, and may even improve this particular explanation. Thus six possible explanations, all suggested at some point in the literature, are considered below. Before continuing it must be noted that I am using Mayr's (1970) general model of allopatric speciation, i.e. involving relatively rapid change under strong directional selection in small peripheral isolates. Carson's (1975; see also Templeton

1980) founder-flush-crash model is not yet considered to have general importance, largely because establishment of a founder population is likely to involve strong natural selection with most founders not succeeding, perhaps even on the Hawaiian islands.

7.3b Reinforcement From Still Other Species

Before considering this idea, the pertinence of the yellow mutants must be elaborated. As noted in Chapter 1, a major reason for the unlikelihood of reinforcement occuring is the complex genetic determination of mating behaviour, particularly with regard to separate control in the sexes. Dow's (1976b) suggestion that the yellow mutation changes both sexes complementarily might indicate that the genetic determinants are the same. As discussed in Chapter 3 this has yet to be conclusively established. It is however the best potential example of this. At best, others, such as that of Hoy et al. (1977), only involve demonstration that both sexes are controlled by sex-linked genes, which is far less precise. In some recent work it has been found that genetic control is on different chromosomes in the two sexes (Grula and Taylor 1980; Zouros 1981), which eliminates the possibility that the genetic determinants are the same. Marler (1976) discussed this idea in terms of shared neural templates for both the generation and recognition of signals, particularly in birds. However, in at least some communication systems, e.g. the tactile recognition system in Enallagma and pheromone systems in many animals, production of the signal is too far removed from its recognition to have the same genetic control. Hence the likelihood of reinforcement being a general phenomenon remains slim.

The suggestion that reinforcement from other species in

allopatry may produce pre-mating isolation from the parental species (e.g. Dobzhansky et al. 1968; Zouros and d'Entremont 1980; Littlejohn 1981) is therefore doubted on the general basis that reinforcement is unlikely. Nevertheless, hybridization may be one of the intense selection pressures placed on a founder population in a new area. Usually this will lead to extinction of the founder or elimination of the cause of the disadvantage, but it is possible that sometimes, for example when considerable divergence already exists, reinforcement might occur.

7.3c Character Displacement to Avoid Interference

As noted in Chapter 1, this is a quite different process from reinforcement. Problems with the two potential examples in the literature (Fouquette 1975; Waage 1979a) were also discussed in Chapter 1. The general idea is that wastage of energy and time, and exposure to predators, should result in selection for differentiation in the first step of the SMRS, as Waage (1979a) feels has occurred in his calopterygid damselflies. This certainly does not generally produce such divergence because in the Enallagma and Platycypha species described here, and probably many other groups (see Chapter 5), males have not evolved the ability to recognise only conspecifics as sexual partners. Littlejohn (1964, 1981) also suggests that, for example in frogs, mating calls, although distinct, might interfere with each other by using the same frequency bands, leading to selection for further divergence. As in the case of ecological character displacement, where intraspecific competition should be greater than interspecific competition if similarity of requirements is the major criterion, so here intraspecific interference should be great if interspecific interfence is to be

sufficiently serious to produce divergence. The general occurence of call alternation in frogs (Wells 1977) may indicate that this is the case, but Passmore and Telford (1981) have found that call alternation does not necessarily facilitate mate location by the females, and may instead function in male-male spacing. Hence interspecific interference is not likely to be great.

As will be argued later, SMRS's can change to fit physical habitats, and features of communication systems are known to evolve in relation to the biotic habitat (e.g. in mimicry and perhaps predator-prey relationships), so this explanation warrants further investigation.

7.3d Founder Effects

Mayr's founder principle has been extended by Kaneshiro (1976, 1980) to suggest that a founder individual may not have a component of the normal SMRS, which would rapidly result in the evolution of a new SMRS. This has been supported by Ohta (1978), but as noted in Chapter 4, Watanabe and Kawanishi (1979) and Wasserman and Koepfer (1980) have questioned the generality of this explanation. Indeed, D. mauritiana appears to have gained, or at least has not lost, aspects of courtship. I have never observed individuals lacking entire aspects of courtship, although particular courtships may not include particular aspects. Instead there would seem to be some variability on all aspects of the courtship and the founder principle should be restricted to chance formation of a new population with a bias in this variability, which might facilitate evolution towards a new SMRS. As a general principle I agree with Watanabe and Kawanishi (1979) that it is more likely that components will be substituted or added than lost.

7.3e Sexual Selection

Used in the broad sense of Darwin (1871), practically any change in SMRS's must be brought about by sexual selection because it would involve differential reproduction. However, this does not tell us much about how it actually happens. Hence I will here restrict the term to characters changed as a result of female choice or preference and especially Fisher's (1930) theory. Fisher (1930) considered his theory to be particularly pertinent for speciation in that it provided a directional stimulus for change. This has been elaborated by Ringo (1977) for the Hawaiian Drosophila, and has been used by Carson (1978) and Templeton (1979). The elaboration of "sexcombs" in a population of D. silvestris on Hawaii may provide an example of this (Carson and Bryant 1979; Spiess and Carson 1981). The idea is that destabilization during speciation may release the run-away process leading to new male traits with a female preference for these, which would constitute a new SMRS. This sort of explanation may well be applied to the evolution of P. caligata from a Chlorocypha-like ancestor, if the assumptions of the run-away process discussed above can be confirmed. It remains to be explained why the run-away process stops and yields to the stability evidenced by SMRS's, because sexual selection is normally thought of as acting continuously, as it should if open-ended female preferences are indeed involved. For example, why do P. fitzsimonsi and the Chlorocypha species not rapidly move towards the elaboration of P. caligata? Why is the change restricted to the punctuated part of the punctuated equilibrium pattern? Furthermore, if the run-away process did involve open-ended female mating preferences, why, for example, do the P. fitzsimonsi females not prefer the P.

caligata males? This entire question is in considerable flux. As Templeton (1979) notes, Paterson's view that the SMRS is normally under strong internal (intraspecific) stabilizing selection may help explain this. Templeton (1979), together with Carson (1978) and Littlejohn (1981), calls this stabilizing sexual selection. This fits Darwin's broad usage, but is perhaps misleading because there is no selection of fitter males, but simply recognition of the norm as a mate. If sexual selection in the narrow sense used here is ever an important force in evolution, it may have considerable importance in speciation, but understanding its replacement by stabilizing selection remains a problem.

7.3f Adaptation to New Environments

There can be little doubt that many signals in the SMRS are in some way related to the environment in which the species lives. This is especially true for distance attraction where light, sound and chemical signals must be transmitted despite impediments such as foliage in the environment. Considerable work has been done on this aspect with vocal communication in birds (e.g. Morton 1975; Marten et al. 1977; Wiley and Richards 1978; Hunter and Krebs 1979), and the structure of frog mating calls can often similarly be related to the environment (Passmore 1981). Hence speciation involving adaptation to a new environment would also involve direct adaptive change of the SMRS. This is a potentially general explanation for the changes in many signals in SMRS's, but our understanding of this depends on an understanding of the functions of individual steps in the SMRS of particular species. Thus, as suggested earlier, the development of colour on the abdomen of the male chlorocyphid damselflies may be related to increased efficiency in leading the

female to the oviposition site in shaded or dark areas. But, for example, whether extent of light dependency in various species of Drosophila is environmentally related cannot be resolved until their general biology is better understood. It is interesting that selection in different light regimes might result in premating isolation (Kilias et al. 1980).

7.3g Pleiotropic Effects

As Mayr (1970) and Wright (1980) have always maintained, pleiotropic effects are generally important in evolution, and they are probably also of considerable importance in promoting changes in SMRS's in allopatry as a byproduct of adaptive change during speciation (Muller 1942; Moore 1957; Mayr 1970). This has sometimes (e.g. Koref-Santibanez and Waddington 1958; Solar 1966; McKenzie and Parsons 1971; Kilias et al. 1980) but not always (e.g. Robertson 1966; Barker and Cummins 1969) been demonstrated in the laboratory when sexual isolation is looked for between lines of Drosophila flies selected for various other differences. When the stabilizing selection entailed by a co-adapted SMRS is considered, it can be added that any pleiotropic change in one sex, if spread against the stabilizing selection by selection for the adaptive change, will lead to stabilizing selection producing an appropriate complementary change in the other sex. Something of this sort can be seen in the close tuning of females to male dialects in some species (e.g. Capranica et al. 1973).

This is the sort of explanation Bastock (1956) offered for the evolution of the yellow D. melanogaster females' receptivity to the mutant males' changed courtship. As discussed in Chapter 3, the applicability of this is not clear, because as yet the genetic

determinants of the females' receptivity cannot be separated from the yellow locus. If they can, then this would provide an excellent example of this process. Examination of other mutants which affect the males' courtship (or the females' receptivity) would be worthwhile.

There are some signal-response systems where it is difficult to see how any other explanation (other than reinforcement) could apply. For example, in the tactile recognition system in Enallagma it is hard to see how any form of interference could be relevant, sexual selection seems hardly applicable, and the signal is not transmitted over any distance, so it cannot be related to the environment.

7.4 Conclusion

Most authors only discuss courtship changes in allopatry when considering interfertile species, but as argued in this thesis, reinforcement is probably not a generally important factor in speciation, so total change in total allopatry should be our general model of speciation. This is particularly so because it applies to the origin of both interfertile and intersterile species. I have tried to show how regarding species and mating behaviour from the positive view of Paterson's recognition concept and the SMRS will allow a better understanding of this process. Various combinations of the above and possibly other explanations will be involved in particular cases, and the further elucidation of these is a major challenge for understanding speciation and evolution.

APPENDIX A. Chemical Stimuli Eliciting Courtship by Males in *Drosophila melanogaster*

Introduction. Volatile chemicals have been implicated in the initiation of courtship by males in *Drosophila melanogaster* (Shorey and Bartell 1970; Averhoff and Richardson 1974; Venard and Jallon 1980; Tompkins et al. 1980). Some authors consider them to be essential (Averhoff and Richardson 1974; Venard and Jallon 1980). Removal of the antennae which have long been considered to be the major olfactory receptors, does not, however, prevent males from courting (Manning 1959b; Hall 1978). Similarly, although contact chemicals gained by the male when tapping the female with his foretarsi are undoubtedly important(Manning 1959b; Spieth 1974), foretarsiless males still court readily (Manning 1959b). Other organs which may receive volatile and/or contact chemicals are the proboscis and the palps (Spieth 1974), and the aristae (Averhoff et al. 1979). To resolve the relative importance of these stimuli in the initiation of courtship, and which organs receive them, males with various combinations of these organs removed were tested for propensity to court females or other males. It is concluded that volatile chemicals are not essential, and perhaps not even sufficient for the initiation of courtship, but contact chemicals perceived via the foretarsi, or the antennae, or even the proboscis in foretarsiless males, probably are essential.

Materials and methods. Four day old Canton-S males were collected after 24 h from standard cultures, kept isosexually 10 per vial, and then singly for 20-24 h before testing. Virgin females were collected after 12 h, kept 10 per vial, and used after a further 12-24 h. The males were operated on under ether

anaesthesia 2 h before testing. Aristae, foretarsi (plus or minus the basitarsi which bear the sexcombs) and proboscides were snipped off with the fine scissors, and palps and antennae were plucked off with forceps . Only the third segment of the antennae, the funiculus, which bears the arista and the olfactory receptors (Averhoff et al. 1979), was removed. Some observations were conducted in red light in a photographic darkroom to remove visual stimuli. Single pairs were observed for 10 min with a binocular dissecting microscope in either side of the standard small perspex observation chamber (see 2.2c) with a double-layer nylon gause partition. This allowed simultaneous observation of two pairs, and the gause provided better purchase for the foretarsiless males. Whether the male courted, as indicated by wing extension and vibration, was noted. Although courtship is not all or none (Nissani 1977) quantitative measures would be unrealistic when comparing these surgically mutilated males.

In the second experiment, test males (normal or foretarsiless) were placed with wingless target males in one half of the chamber, first without and later with 10 virgin females in the other half. The amount of orientation and wing vibration by the test males was measured simultaneously on stopwatches for 5 min in each condition.

Results and discussion. Initial results confirmed that amputation of either the aristae (1), the antennae (2), the palps and probosicis (3), or the entire foretarsi (4) does not prevent males from courting (Table 28). Although visual stimuli are used in the initiation of courtship, they are not essential in D. melanogaster (Connolly et al. 1969; Hall et al. 1980). Even when removed together with the antennae (5) or the foretarsi (6), courtship was not prevented. Progressive removal of all these

Table 28. Effect of various amputations on the propensity of D. melanogaster males to court females. N_1, number of pairs observed; N_2, number in which male courted; +, intact; -, amputated.

	Light	Aristae	Antennae	Palps	Proboscis	Foretarsi	Basitarsi	N_1	N_2
1	+	-	+	+	+	+	+	5	5
2	+	+	+	-	-	+	+	5	5
3	+	+	+	+	+	-	-	5	5
4	+	-	-	+	+	+	+	10	10
5	-	-	-	+	+	+	+	10	10
6	-	+	+	+	+	-	-	10	10
7	+	-	-	-	-	+	+	5	4
8	-	+	+	-	-	-	-	5	5
9	+	-	+	+	+	-	-	10	9
10	+	-	-	+	+	-	+	5	4
11	+	-	+	-	-	-	-	10	10
12	+	-	-	-	-	-	+	10	9
13	+	-	-	+	+	-	-	15	2
14	+	-	-	-	+	-	-	10	1
15	+	-	-	+	-	-	-	10	0
16	+	-	-	-	-	-	-	10	0

organs (7-10) suggested that the foretarsi or antennae and aristae alone are sufficient for the males to court. Indeed, males with only antennae (11) or only the basitarsi (12) courted fairly readily

Table 28. Effect of various amputations on the propensity of D. melanogaster males to court females. N_1, number of pairs observed; N_2, number in which male courted; +, intact; -, amputated.

	Liy..	Aris-tae	Anten-nae	Palps	Probo-scis	Fore-tarsi	Basi-tarsi	N_1	N_2
1	+	-	+	+	+	+	+	5	5
2	+	+	+	-	-	+	+	5	5
3	+	+	+	+	+	-	-	5	5
4	+	-	-	+	+	+	+	10	10
5	-	-	-	+	+	+	+	10	10
6	-	+	+	+	+	-	-	10	10
7	+	-	-	-	-	+	+	5	4
8	+	+	+	-	-	-	-	5	5
9	+	-	+	+	+	-	-	10	9
10	+	-	-	+	+	-	+	5	4
11	+	-	+	-	-	-	-	10	10
12	+	-	-	-	-	-	+	10	9
13	+	-	-	+	+	-	-	15	2
14	+	-	-	-	+	-	-	10	1
15	+	-	-	+	-	-	-	10	0
16	+	-	-	-	-	-	-	10	0

organs (7-10) suggested that the foretarsi or antennae and aristae alone are sufficient for the males to court. Indeed, males with only antennae (11) or only the basitarsi (12) courted fairly readily

and continuously. Some males with only proboscis and palps (13) or only a proboscis (14) courted, but males with only palps (15) or all the organs removed (16) never courted. These last males (16) suffered minimally more operative trauma than those with only basitarsi (12), yet the latter courted readily, so it can be concluded that lack of courtship was not simply due to operative trauma. Also, the males were not simply courting in the absence of stimuli.

Males in every category usually tapped the female (for example, 6 of the 10 males with all the organs removed tapped), so volatile chemicals, unless received by still another organ, are unnecessary for tapping. Males with only basitarsi courted readily (12), so volatile chemicals are also unnecessary for courtship, unless they are received via the basitarsi or the sexcombs. The latter are mechanical aids in grasping the female's abdomen during copulation attempts (Cook 1977), and do not appear to be sensory organs. Although the basitarsi do not appear to bear olfactory receptors, this possibility should be investigated further.

In all the tests with considerable amputation the males were also tested with 3 day old males, either immediately before or after testing with the female. Most tapped the male, but almost all either did not court, or, especially after courting the female, started courting but stopped within the 10 min observation period after tapping vigorously. The only exception was one of the two antennaeless and foretarsiless males which courted the female (13), and then did not stop courting the male within 10 min. Thus tapping is not sex-specific, and males apparently either do not release the stimuli which normally elicit courtship of females, or

release different stimuli in the same modalities.

Males with only their antennae courted the females (11) but not the males, so volatile chemicals, although unnecessary, might be considered to be sufficient to elicit courtship, and contact chemicals might be unnecessary. However, these males initially tapped the female or male with their foretibiae and then pressed their antennae against the other fly. Those with both antennae and foretarsi removed (Jallon and Hotta 1979; Hall et al. 1980) sometimes licked the female with their proboscides. Males with only the basitarsi remaining (Cook 1977; Nissani 1977) did not do this, which suggests that it was not simply the result of closer contact of the male with the female, but rather an action substituting for the loss of contact chemicals normally received via the foretarsi. In another melanogaster-group species, D. malerkotliana, the males apply their antennae to the females' genitalia in an action analogous to normal D. melanogaster licking prior to attempted copulation (Narda 1966). It is therefore possible that foretarsiless males obtain the appropriate contact chemicals via their antennae, or sometimes eventually via the proboscis. I therefore conclude that volatile chemicals may not even be sufficient for the initiation of courtship, but contact chemicals are probably essential.

The importance of contact chemicals should not be underestimated, as has sometimes been the case (Nissani 1977; Hall 1978; Jallon and Hotta 1979; Hall et al. 1980). Indeed, perception of contact chemicals may explain most of the recent experiments ostensibly demonstrating the significance of volatile chemicals (Venard and Jallon 1980; Tompkins et al. 1980; Tompkins and Hall 1981), because the males had the opportunity to touch the extracts

from the females or males. Only one experiment by Tompkins et al. (1980) involved physical separation of the males from the extract. In a similar experiment I did not find the significant increase in courtship of wingless target males by test males (even when foretarsiless) in the presence of 10 females in the other half of the chamber (Table 29).

Table 29. Amount of courtship directed by normal or foretarsiless males at wingless target males in 5 min tests in the absence or presence of 10 females. The student's t-test for related samples was used to compare means.

Parameter	Test male	N	Control	Females	t	p
Orientation	Normal	15	2.9 ± 3.0	9.0 ± 2.9	1.68	N.S.
	Foretarsiless	10	10.2 ± 3.8	10.3 ± 3.4	0.12	N.S.
Vibration	Normal	15	0.5 ± 0.6	0.7 ± 0.7	1.10	N.S.
	Foretarsiless	10	0.9 ± 1.0	1.0 ± 0.9	0.43	N.S.

Since Tompkins et al.(1980) were unable to repeat the earlier olfactometry experiments (Shorey and Bartell 1970; Averhoff and Richardson 1974), the evidence for the involvement of volatile chemicals in the initiation of courtship remains slender, and the present results further question their importance.

APPENDIX B. Female Preference for Normal or Wingless Males in D. melanogaster

Eastwood and Burnet (1979) found that when presented with the choice of a normal and a wingless male, single Novosibirsk strain females mated randomly. Averhoff and Richardson (1974), without presenting experimental techniques or data, reported similar results. In the light of the clearcut disadvantage of wingless males in single-pair matings (e.g. Manning 1967a) and in mass-matings (e.g. Ewing 1964) this result is suprising. I therefore repeated this experiment with Canton-S males which are established here (2.3d) as being extremely disadvantaged when wingless in single-pair matings.

Single 3 day old females were placed in the small perspex observation chamber with a normal and a wingless male (both 3 days old; wings removed on day 2) and observed till copulation or for 30 min. The normal male copulated in 36 of the 46 trials, the wingless in 4, and 6 did not lead to copulation. Unlike those of Averhoff and Richardson (1974) these wingless males were highly competent in all aspects other than final successful copulation, i.e. they usually started courting first and usually ousted the normal male from the position behind the female. The males' jostling for position behind the female appeared to agitate and inhibit her so that many trials were prolonged before copulation. The winged male usually copulated when briefly courting the female alone. It seemed that a single, undisturbed, normal courtship bout was sufficient and important for copulation.

Clearly the wingless males' disadvantage is retained in these results, which strongly support the contention that, at least in this strain, the wing vibration provides important, even if not essential, stimuli. Eastwood and Burnet's (1979) suggestion

that the importance of these stimuli differs in various strains best explains these, their, and Averhoff and Richardson's (1974) results.

APPENDIX C. Courtship Duration Decreases With Age in Young D. melanogaster Females

Manning (1967a) found that young D. simulans females, if receptive, exhibited uniformly short courtship durations before

Table 30. Change in number of females mating and courtship duration with female age.

		Courtship duration (s)	
Female age (h)	N	Mean	S.D.
12 - 15	0	-	-
15 - 18	0	-	-
18 - 21	2	914	795
21 - 24	3	430	285
24 - 27	9	550	445
27 - 30	-	-	-
30 - 33	8	385	297
33 - 36	-	-	-
36 - 39	10	288	187
39 - 42	8	395	239
42 - 45	10	297	183
45 - 48	8	314	277
48 - 51	10	186	92
51 - 54	-	-	-
54 - 57	10	207	155
78 - 81	10	194	200

mating, suggesting that switch-on of female receptivity is independent of the "female courtship summation process" which ostensibly controls courtship duration. Yacher and Spiess (1973) and Cook (1973a) independently found that mating speed and courtship duration increased and decreased respectively, in D. persimilis and D. melanogaster, suggesting that switch-on and courtship duration are not independent and both are controlled by the female courtship summation threshold.

I collected Canton-S females every hour and tested them in single-pair matings with 3 day old males which had been kept singly for 1 day. The tests were done in the small perspex chamber between 0800 and 1100 h, so that females could be categorised in 3 h brackets, for 30 min each with 10 females tested per 3 h bracket. Most females were receptive after 24 h, but courtship duration continued to decrease until 48 h, after which it was constant (Table 30). These results agree with those of Yacher and Spiess (1973) and Cook (1973a). This effect may however only be the result of a decrease in the length of the "female latency" resulting from the females' agitation (Chapter 2). Eastwood and Burnet (1977) found such an effect for the male latency to courtship. Switch-on of female receptivity may yet be shown to be independent.

APPENDIX D. Polygenic Autosomal Control of Male Genitalic Shape in D. simulans and D. mauritiana

The study of the genetics of species differences is usually limited by the prevalence of hybrid sterility. Thus species pairs which produce fertile hybrids, and exhibit structural or behavioural differences, are particularly useful for such studies. Drosophila simulans and D. mauritiana produce fertile female hybrids (David et al. 1974), and differ clearly in the shape of the posterior lobe of the genital arch of the male genitalia. This structure is large and flat with a large ventral hook in simulans and narrow and straight in mauritiana (Fig. 28).

Table 31. Shape of the posterior lobes of the genital arches in male backcross progeny of hybrids of D. simulans and D. mauritiana.

F_1 female Male	simQ-mauσ mau	mauQ-simσ mau	simQ-mauσ sim	mauQ-simσ sim	Total
F_1	39	44	34	44	161
Intermediate	108	136	117	110	471
Parental	52	51	43	73	219

In hybrids from both reciprocal crosses (240 males examined) this structure was uniformly intermediate, i.e. midway in breadth with a small ventral hook. This suggests autosomal control with no dominance. The backcross progeny showed continuous variation from this F_1 shape to the shape of the appropriate male parent. They were therefore broadly classed as being F_1, intermediate or







Figure 28. Scanning electron micrographs of the male genitalia. The anal plates are above with the genital arches below. The posterior lobes protrude centrally. D. simulans (top) has large lobes, D. mauritiana (middle)has thin slender lobes, and for comparison D. melanogaster (bottom) has intermediate sized lobes (each 100X).

male parental (Table 31). At least two independently assorting loci must be involved since the ratios approximate 1 : 2 : 1 proportions. the continuous variation which is artificially compartmentalised in this classification suggests that more loci are involved. Again there is no evidence of dominance or sex linkage.

Figure 28. Scanning electron micrographs of the male genitalia. The anal plates are above with the genital arches below. The posterior lobes protrude centrally. D. simulans (top) has large lobes, D. mauritiana (middle)has thin slender lobes, and for comparison D. melanogaster (bottom) has intermediate sized lobes (each 100X).

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APPENDIX E. Effect of Age and Sexual Deprivation on Courtship of *D. mauritiana* Females by *D. melanogaster* Males

Few studies have been made on the effect of age and sexual deprivation on the specificity of courtship responses in *Drosophila*. Pruzan et al. (1979) have shown that virgin females of some *D. paulistorum* "semispecies" and the sibling species *D. melanogaster* and *D. simulans* aged 9 and 11 days respectively are usually as unlikely to mate interspecifically as are 3 day old virgins. What about the propensity of males to court interspecifically?

I compared the propensity of 2 and 12 day old *melanogaster* males to court 3 day old *mauritiana* females. For each age class 20 single pairs were observed in the small perspex observation chamber for 10 min each. Of the 2 day old males, 6 only tapped the female and then left her. Another 3 started courting by following and directing wing vibration at the female, but eventually tapped her again and stopped courting. The remaining 11 continued courting until they licked the female, and then stopped after tapping her vigorously. Thus within 10 min all 20 2 day old males stopped courting the *mauritiana* females.

Of the 12 day old males, 1 stopped courting after licking the female and another 3 after making full attempts at copulation. The remaining 16 males courted throughout the 10 min observation period and 6 attempted copulation (they had some difficulty because the females avoided them by running). Clearly age and/or sexual deprivation reduced the specificity of the males' responses. Further experiments with old but non-deprived males are needed.

APPENDIX F. Male Activity During Drosophila Copulation

Drosophila flies are always described as being inactive or quiescent during copulation. Thus despite numerous reports of measurements and comparisons of copulation durations, Spieth's (1952) comment that "... copulation usually proceeds without any considerable action on the part of either partner" remains applicable.

During the experiments reported in this thesis on the Canton-S strain of D. melanogaster, a locally caught strain of simulans, and a D. mauritiana strain, I noticed that during copulation in all three species the male repeatedly extended and vigorously rubbed or vibrated his meso- and metathoracic legs against the sides of the female's thorax and legs. The mesotarsi in particular were rubbed against her thorax and often reached onto her eyes. In melanogaster these bursts of rubbing were initially brief (± 0.5 s) and frequent (up to 5 per min) and later became longer (± 1 s) and less frequent (about 1 per min). Each copulation contained 25.4 ± 9.9 (S.D. ; N = 10) bursts of rubbing (copulation duration = 17.2 ± 2.2 min).

To investigate whether this rubbing somehow enhanced fertility, perhaps by promoting sperm transferal, single melanogaster females were placed in food vials, each with two males with various degrees of amputation of the meso- and metathoracic legs. The vials were examined for progeny after twenty days. Removal of meso- and metatarsi (N = 15), meso- and metatarsi and mesotibiae (N = 30), or meso- and metatarsi and tibiae (N = 20) did not have any obvious qualitative effect since most vials contained large numbers of progeny. In each test a few vials contained no progeny, presumably because the

males were unable to copulate.

Observations of a few copulations by these amputated males showed that they still attempted to rub the female's thorax, but, especially in the last test, their legs did not reach further than her abdomen. These copulations were also of normal length. Dissection of females inseminated within the last 24 h by these latter males showed no obvious difference in the sperm content or placement in the seminal receptacle or spermathecas when compared to females similarly inseminated by normal males.

The initially high frequency of rubbing while the female makes what appear to be rejection movements, e.g. kicking and pushing with the hindlegs, might suggest that the rubbing induces female quiescence, but later when the pair was quiet, the males' vigorous rubbing instead induced female activity. Also, females were as quiet with amputated males. Therefore, as yet, no function can be ascribed to this, in fact, prominent male activity during copulation.

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