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Genetic studies of three sibling species of *Drosophila* with relationship to theories of speciation

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SUMMARY

Drosophila melanogaster, *D. simulans* and *D. mauritiana* are closely related species, the first two cosmopolitan and the last restricted to the oceanic island of Mauritius. *D. simulans* and *D. mauritiana* are the most closely related pair, with the latter species probably resulting from a founder event. The relatedness of the three species and their ability to hybridize allow tests of recent theories of speciation. Genetic analysis of two characters differing between *D. simulans* and *D. mauritiana* (sex comb tooth number and testis colour) show that the differences are due to at least five and three loci respectively. Behavioural tests further demonstrate that sex combs are probably used by males at a crucial step in mating, and that the differences between the two species may be related to differences in their mating ability. These genetic studies and previous work indicate that differences among these species are polygenic and not (as proposed by recent theories) attributable to only one or two loci of large effect. Further studies of interspecific hybrids show that genetic divergence leading to developmental anomalies is more advanced in the older species pair *D. simulans*/*D. melanogaster* than in the younger pair *D. simulans*/*D. mauritiana*. This supports the neo-Darwinian contention that reproductive isolation is one step in a continuous process of genetic change among isolated populations, and does not support current theories that such change occurs only during the evolution of reproductive isolation. Finally, investigations of the degree of gonadal atrophy and its sensitivity to temperature in *D. simulans*/*D. mauritiana* hybrids fail to support recent speculations that phenomena similar to hybrid dysgenesis (which causes such atrophy in *D. melanogaster*) play a role in speciation.

1. INTRODUCTION

Despite half a century of interest in and speculation about speciation, virtually nothing is known of the genetic changes occurring when one species becomes two. The frequent separation of related species pairs by geographic barriers has led to the conclusion that physical isolation of populations is important in speciation, but the genetic processes leading to reproductive isolation are not so easily inferred from geography. Island populations of birds, for example, often diverge in morphology from mainland populations, although geographic distances between

mainland populations may be greater. This observation led Mayr (1954, 1963) to propose that island speciation results from novel genetic processes ('genetic revolutions') involving genetic drift in small colonizing populations. Yet another interpretation is possible: speciation on islands involves no genetic revolution but is merely the byproduct of adaptations accelerated by novel island habitats and the lack of gene flow normally retarding differentiation among populations. Conflicting interpretations of identical data are common in the speciation literature, making it unlikely that biogeography will shed much light on the genetics of species formation.

One way around this difficulty is to infer the process of speciation from the *genetic* pattern of species differences. Recent theories have produced testable predictions about how the genetic basis of differences among species may be influenced by different modes of speciation. These theories lead to the following questions:

(A). *What is the genetic basis of morphological and reproductive differences among related species*? Templeton (1981, 1982) proposed that speciation involving genetic drift in small populations would lead to differences among species based on one or a few allelic substitutions of large effect. Wright (1982) also postulated that monogenic character differences would be associated with the occupation of new niches, as might occur during island colonization. Advocates of the theory of punctuated equilibrium have suggested that single mutations of large effect may also be important in speciation and macroevolution (Stanley, 1979; Gould, 1977, 1980). Classical theories of geographic speciation propose, on the other hand, that species differences result from the accumulation of many allelic differences of small effect (Charlesworth, Lande & Slatkin, 1982). Previous work on animal species strongly supports the polygenic hypothesis (Coyne, 1983). Some of this evidence is questionable, however, because it is based on species that may have been isolated for long periods, so that the described genetic differences may have accumulated after speciation. More studies – particularly on species separated by founder events – are needed to test the generality of the polygenic theory of species differences.

(B). *How do genetic differences among populations accumulate with respect to the origin of reproductive isolation*? The theory of punctuated equilibrium predicts that genetic differentiation among related species arises primarily during the speciation event, with little change occurring thereafter. An identical prediction arises from Carson's (1975) theory of founder-event speciation, which posits a restructuring of a usually 'closed' system of epistatically interacting genes during speciation, and relatively little gene substitution thereafter. These theories would predict that genetic change among related species is proportional to the number of speciation events separating them and independent of the absolute time since they diverged. Although this prediction is violated by electrophoretic data (Ayala, 1975; Avise, 1976), defenders of this view assert that allozyme substitutions are neutral and that the genetic analysis important in testing their theory must involve loci affecting morphology, physiology and development (Gould, 1980).

(C). *Do novel genetic phenomena such as transposable elements contribute to speciation*? Hybrid dysgenesis in *Drosophila melanogaster* is a complex syndrome of elevated mutation rate, segregation distortion, male recombination, sterility and

temperature-sensitive gonadal dysgenesis that occur in hybrids between some strains (Kidwell, Kidwell & Sved, 1977; Sved, 1979; Engels & Preston, 1979; Bingham, Kidwell & Rubin, 1982; Bregliano & Kidwell, 1983). Dysgenesis is often found in the offspring of a father recently derived from nature and a mother from an older laboratory strain. At least part of this syndrome is caused by the presence of transposable, repeated segments of DNA in wild strains that infect the genome and produce aberrant effects in genetically sensitive strains (Kidwell, 1983). It has been proposed that such dysgenesis may play a role in speciation by causing differential loss and accumulation of either the mobile DNA elements or genetically determined sensitivity to them in geographically isolated populations. This could cause reproductive disharmony in inter-population hybrids (Engels & Preston, 1979; Bregliano & Kidwell, 1983; Kidwell, 1983; Rose & Doolittle, 1983; Ginzburg, Bingham & Yoo, 1984).

Here I investigate these three questions using genetic analysis of three sibling species of *Drosophila* of different evolutionary relatedness. The divergence between two of these species almost certainly involved a founder event.

The species. Females of the three sibling species *Drosophila melanogaster*, *D. simulans* and *D. mauritiana* are morphologically identical, but males can be distinguished by the shape of their genital arch (Sturtevant, 1919; Tsacas & David, 1974). *D. simulans* and *D. melanogaster* are cosmopolitan human commensals, while *D. mauritiana* lives only on the 2040 km^2 volcanic island of Mauritius, 1000 km east of Madagascar in the Indian Ocean. Neither *D. simulans* nor *D. melanogaster* inhabits Mauritius (Tsacas & David, 1974).

Several methods have clarified the evolutionary relationships of these species. *D. simulans* and *D. mauritiana* are identical in polytene chromosome banding pattern, but both differ from *D. melanogaster* by at least ten rearrangements (Horton, 1939; Lemeunier & Ashburner, 1976). *D. simulans* and *D. mauritiana* can be crossed easily to yield fertile hybrid females and sterile hybrid males. Both species, however, give only sterile unisexual progeny in a more difficult cross to *D. melanogaster* (Sturtevant, 1920; David *et al.* 1974). Further studies of these species using allozyme electrophoresis, two-dimensional electrophoresis, restriction endonuclease patterns, satellite and mitochondrial DNA analysis, and sequencing of the alcohol dehydrogenase gene confirm that *D. mauritiana* is very closely related to *D. simulans*, with both of these species more distantly related to *D. melanogaster* (Barnes, Webb & Dover, 1978; Coen, Strachan & Dover, 1982; Gonzales *et al.* 1982; Strachan *et al.*, 1982; Ohnishi, Kawanishi & Watanabe, 1983; Cohn, Thompson & Moore, 1984; Bodmer & Ashburner, 1984).

The biogeography and evolutionary relationships of this group strongly imply that *D. mauritiana* arose after colonization of Mauritius by proto-*simulans* founders that had already diverged from the *D. melanogaster* line. Thus the partial fertility of *D. mauritiana*/*D. simulans* hybrid females affords an opportunity to study genetic differences among species related by a founder event, and to compare these with differences between the more distantly related species pair *D. simulans* and *D. melanogaster*. The following genetic studies of these three species addressed the three questions given above.

Genetic basis of morphological differences. Previous studies (Coyne, 1983, 1984,

1985) showed that differences between *D. simulans* and *D. mauritiana* in genital morphology and hybrid fertility were caused by substitutions at a minimum of five and six loci respectively, supporting a polygenic basis of the species differences. Two additional characters are now known to distinguish these species: the colour of the testis (David *et al.* 1976) and the number of teeth in the sex comb (a special row of enlarged bristles found on the male tarsus in some *Drosophila* species). Here I analyse the genetic basis of the interspecific differences in these characters to determine the numbers and effects of genes diverging after a founder event. Other work was undertaken to understand the function of the sex comb and the significance of the difference in tooth number between the species.

Developmental anomalies in species hybrids. Although these three species are almost morphologically identical, hybrids between *D. melanogaster* and *D. simulans* show various developmental anomalies including distorted sex ratios, gonadal atrophy, and abnormal bristle patterns and abdominal chitinization (Sturtevant, 1929; Biddle, 1932; Weisbrot, 1963). These anomalies mean that morphologically similar characters in the two species are actually the products of different alleles. Such genic divergence may reflect divergent selection for genes having a pleiotropic effect on morphology, combined with stabilizing selection on the unchanged morphological character (Weisbrot, 1963). These changes between what may be adaptive morphological peaks are facilitated by the combination of selection and genetic drift occurring in small populations (Carson, 1975; Wright, 1982). This study investigated the possibility of similar anomalies in hybrids of *D. simulans* and *D. mauritiana*. If such genic divergence is indeed concentrated in the speciation event and enhanced in founder populations, one might expect *D. simulans*/*D. mauritiana* hybrids to show developmental disharmony similar in degree to that of *D. simulans*/*D. melanogaster* hybrids. A theory of speciation by gradual adaptive divergence would predict, on the other hand, that such developmental disharmonies result from genetic differences accumulating continuously with time, and would be less severe in *D. simulans*/*D. mauritiana* hybrids.

Gonadal dysgenic sterility. Crosses were made to determine if gonadal atrophy reported in hybrids between *D. melanogaster* and *D. simulans* also occurs in hybrids of the latter species with *D. mauritiana*, and whether any gonadal atrophy is temperature-sensitive, as it is in P–M dysgenic crosses within *D. melanogaster* (Engels & Preston, 1979; Kidwell & Novy, 1979; Schaefer, Kidwell & Fausto-Sterling, 1979).

2. METHODS, MATERIALS AND RESULTS

All flies in the following studies were reared at 23° under a 16 h/8 h light/dark cycle.

(i) *Genetic analysis of sex comb tooth number*

After preliminary inspection indicated a difference in sex comb tooth number between males of *D. simulans* and *D. mauritiana*, a systematic survey was made of geographic lines of these species and their relative *D. melanogaster*. Recently collected strains (most of them isofemale) were studied by removing forelegs from

males raised in uncrowded vials and counting sex comb teeth with a compound microscope. Table 1 shows a consistent difference in this character between *D. mauritiana* on the one hand and *D. simulans* and *D. melanogaster* on the other. Although there is significant heterogeneity among strains within each species (*simulans*: $G_{30} = 57{\cdot}7$; *mauritiana*: $G_{56} = 233{\cdot}8$; *melanogaster*: $G_{20} = 67{\cdot}8$; all $P < 0{\cdot}005$), there is very little difference between the mean tooth number among strains of *D. simulans* (10·14) and of *D melanogaster* (10·17). Both, however, differ from the mean tooth number of *D. mauritiana* strains (13·56).

Table 1. *Sex comb tooth number of* D. melanogaster, D. simulans *and* D. mauritiana strains

Strain	*n*	Mean tooth number	Standard error
	*D. mauritiana**		
Bowling Green	200	14·32	0·08
72	50	13·80	0·16
75	50	13·66	0·16
95	50	14·64	0·14
102	50	12·22	0·16
152	50	13·08	0·15
197	50	12·88	0·15
206	50	13·76	0·16
	D. simulans		
Beltsville, MD	50	9·96	0·15
Cairns, Australia	50	9·94	0·14
Davis, CA	50	10·40	0·17
Kenya	50	10·42	0·12
LHR (Japan)	50	10·48	0·12
Oxnard, CA	200	9·86	0·06
	D. melanogaster		
Bahia, Brazil	50	9·48	0·13
Greenbelt, MD	50	10·46	0·15
Lakeside, CA	50	9·64	0·17
Szedag, Hungary	50	10·66	0·13
Victoria, Australia	50	10·48	0·13

*All numbered strains are isofemale lines derived from flies captured on Mauritius in 1981 by O. Kitagawa.

Estimates of the number of gene substitutions responsible for the tooth-number difference between *D. simulans* and *D. mauritiana* were derived from a backcross analysis using genetically marked segments of *D. simulans* chromosomes. This classical genetic technique showed previously that at least five allelic substitutions were responsible for hybrid sterility and the differences in male genital morphology among the species (Coyne, 1983, 1984). The cross uses a *D. simulans* stock homozygous for the recessive mutants f^2; *nt pm; st e*, with *forked-2* on the *X* chromosome (f^2: *I*-60), *net* and *plum* on the two arms of the second chromosome (*nt*: II-0, 2L; *pm*: II-103, 2R), and *scarlet* and *ebony* on the two arms of the third chromosome (*st*: III-44, 3L; *e*: III-71, 3R). Females of this stock were crossed to males from the Bowling Green strain of *D. mauritiana* used in previous studies.

Female interspecific F_1 hybrids were backcrossed to *D. simulans* f^2; *nt pm*; *st e* males, and the segregating male backcross progeny scored for sex comb tooth number. Ten of the 32 possible backcross classes were chosen for analysis, so that the effect of each chromosome or chromosome arm on the character could be tested in at least two independent comparisons between pairs of genotypes (see Coyne, 1983, for a further description of the crossing scheme and method of analysis). An independent test of the effect of the *X* chromosome was made by comparing offspring from reciprocal crosses between the *D. mauritiana* Bowling Green stock and a wild-type stock of *D. simulans* from Oxnard, California (males of the multiple marker stock would not mate with *D. mauritiana* females). Male offspring of the two crosses differ genetically only by the source of *X* and *Y* chromosomes and the species donating egg cytoplasm.

Table 2. *Backcross analysis of male sex comb tooth number in hybrids between* D. simulans *and* D. mauritiana

Genotype	*n*	Mean tooth number	Standard error
Parental species			
D. simulans f^2; *nt pm*; *st e*	100	9·30	0·08
D. mauritiana Bowling Green	200	14·31	0·08
F_1 hybrids			
(a) maur. BG ♀ × sim. Oxnard ♂	103	12·33	0·10
(b) maur. BC ♂ × sim. Oxnard ♀	200	12·10	0·07
Backcross males			
(1) f^2; *nt pm*; *st e*	100	9·91	0·10
(2) *nt pm*; *st e*	100	10·03	0·10
(3) f^2; *nt*; *st e*	100	10·28	0·10
(4) f^2; *pm*; *st e*	100	10·25	0·16
(5) f^2; *nt pm*	100	10·86	0·11
(6) f^2; *st e*	100	10·66	0·08
(7) f^2; *st*	49	11·14	0·15
(8) f^2; *e*	41	10·90	0·12
(9) f^2	100	11·63	0·09
(10) + (wild type)	100	11·90	0·10

Table 2 gives the results of this series of crosses and Table 3 the statistical analysis of the main effects of chromosomes and arms. These effects were tested by comparing the means of a pair of genotypes differing only in the presence of the relevant recessive alleles. The effect of the second chromosome, for instance, can be gauged by comparing the tooth number of f^2; *nt pm*; *st e* males with that of f^2; *st e* males, or of f^2; *nt pm* males with f^2 males. Such pairwise comparisons were made using *t* or *t'* tests depending on the significance of variance ratios (Sokal & Rohlf, 1981). The *a priori* hypothesis that segments of *D. simulans* genome would reduce the number of sex comb teeth dictated one-tailed tests of significance.

The effect of the *X*-chromosome is significant in two of the three individual tests. Amalgamating the result of all three according to Fisher's method of combining probabilities from independent tests of significance (Sokal & Rohlf, 1981), one finds the overall effect of the *X* chromosome highly significant ($-2 \Sigma \ln P = -17{\cdot}39$,

6 D.F., $P < 0{\cdot}01$). Although the reciprocal crosses differ in the source of cytoplasm, the pairs of genotypes compared in the backcross do not. It is therefore unlikely that the cytoplasm plays a major role in the character difference.

The effect of the second chromosome is significant in both comparisons, as are all comparisons for the effects of the right and left arms. This chromosome must carry at least two genes affecting the character difference.

Table 3. *Statistical analysis of sex comb tooth number in backcrosses between* D. simulans *and* D. mauritiana

(Original data as well as numbers and letters of compared genotypes are taken from Table 2. t tests were used for comparison unless significant variance ratios indicated the use of t' tests. The bottom three comparisons measure the relative effects of the two major autosomes and the two arms of each autosome.)

Chromosome or arm tested	Genotypes compared	Difference in means	t or t'	DF	One-tailed P
X	(a) vs. (b)	0·23	1·87	300	0·03
	1 vs. 2	0·12	0·85	198	0·20
	9 vs. 10	0·27	1·94	198	0·03
2 entire	1 vs. 6	0·75	5·58	198	<0·001
	5 vs. 9	0·77	5·00	198	<0·001
3 entire	1 vs. 5	0·95	6·20	198	<0·001
	6 vs. 9	0·97	7·73	198	<0·001
2 left arm	1 vs. 4	0·34	2·19	198	<0·02
	3 vs. 6	0·38	2·95	198	<0·01
2 right arm	1 vs. 3	0·37	2·61	198	<0·01
	4 vs. 6	0·41	2·85	99	<0·01
3 left arm	6 vs. 8	0·24	1·51	139	0·066
	7 vs. 9	0·49	3·03	147	0·001
3 right arm	6 vs. 7	0·48	3·01	147	<0·005
	8 vs. 9	0·73	4·44	139	<0·005
2 vs. 3	5 vs. 6	0·20	1·41	99	>0·10*
2L vs. 2R	3 vs. 4	0·03	0·20	198	>0·50*
3L vs. 3R	7 vs. 8	0·24	1·24	41	>0·20*

*Two-tailed probabilities.

The effect of the third chromosome on tooth number is significant in both independent tests, as is the effect of the right arm alone. The left arm has a significant effect in one of the two tests, and an overall significant effect when the results of both tests are combined using Fisher's test ($-2 \Sigma \ln P = -18{\cdot}79$, 4 D.F., $P < 0{\cdot}05$). There are thus at least two loci on the third chromosome affecting the interspecific difference in tooth number.

Table 2 also gives the average effect of each chromosome and arm on the character difference. The two large autosomes have roughly equal effects, while the *X* chromosome, which is half the size of either autosome, has the smallest. There is no significant difference between the effects of the two autosomes, nor between the two arms of either autosome.

In sum, at least five gene substitutions are responsible for the difference in sex

comb tooth number between the *D. mauritiana* and *D. simulans* strains. This is the largest number of loci that could have been detected by this method, and implies that the true genic divergence is even greater. It is obvious, then, that the interspecific character difference is not due to only one or two loci of large effect. Because the tooth number of the *D. mauritiana* strains in these crosses is at the higher end of this species' range of the character, it is possible that some of the loci implicated in this analysis are actually polymorphic within *D. mauritiana*. This is impossible to determine without localizing the genes and measuring their effect on the character. Nevertheless, the lack of evidence in these crosses for any loci of large effect, the roughly equal effect of all chromosome arms, and the lack of bimodality in the distribution of bristle numbers in independent backcrosses, indicates that the difference between the species is almost certainly polygenic.

(ii) *Meaning of the character*

What is the significance of the difference between these two species in sex comb tooth number? The answer is probably related to the function of the character itself. The limitation of sex combs to males immediately suggests a sexual role. Spieth (1952) proposed that sex combs of *D. pseudoobscura* help males spread females' wings before mounting and copulation. My observations of mating *D. simulans* and *D. mauritiana* showed that the sex combs do not touch the female wing before or during mating. Cook (1977) found that removing sex combs of *D. simulans* or *D. melanogaster* males delays but does not entirely suppress copulation, and suggested that these combs help males grasp the female genitalia before mating. Other possible functions of sex combs are release of pheromones, tactile stimulation of females, and reception of chemical signals from females.

In experiments similar to those of Spieth (1936), I amputated various segments of the legs of male *D. mauritiana* and *D. simulans* and tested these treated males for their ability to inseminate conspecific females.

The first strain studied was the Bowling Green strain of *D. mauritiana*. Four-day-old virgin males were subjected to one of four treatments under CO_2 anaesthesia: (1) control treatment (flies anaesthetized, but no operation performed); (2) both foretarsi clipped immediately above the sex comb, removing this structure; (3) both foretarsi clipped immediately below the sex combs, retaining the structure; and (4) one leg clipped immediately above the sex comb, with the other leg left intact. The only difference between treatments (2) and (3) is the presence on both legs of the tarsal segment containing the sex comb. Immediately after the operation, groups of five identically treated males were placed in food vials with 10 four-day-old virgin females of the same species. In this first study, vials were left at 23° for 24 h, and then females were dissected in Ringer's solution and inspected for the presence of transmitted sperm. Table 4 gives the results for all four treatments. Males subjected to treatments (1), (3) and (4) inseminated at least 86% of females, but males given treatment (2) (removal of both combs) inseminated only 5% of the females. The significant heterogeneity among these treatments ($G_3 = 300{\cdot}0$, $P < 0{\cdot}001$) is removed if treatment (2) is excluded ($G_2 = 4{\cdot}06$, n.s.). Apparently the presence of at least one sex comb-containing

segment is necessary for insemination of *D. mauritian* females, as the amputation itself (treatment 3) had no effect on insemination frequency.

Treatments (2) and (3) only were repeated on a 'mixed' stock of *D. mauritiana* made by combining six isofemale strains collected by O. Kitagawa in 1981. The results (Table 4) are similar to those of the previous test: males missing both sex comb-containing tarsal segments have a very poor ability to inseminate females compared to amputated flies possessing combs ($G_1 = 159{\cdot}7$, $P < 0{\cdot}001$), and there is little effect of leg amputation itself on the ability to inseminate females.

Table 4. *Number of females inseminated when mated to treated males.*

(Thirteen replicate tests were conducted, each using five males and ten virgin females. Treatments were as follows: 1, control (anaesthesia but no operation); 2, leg amputated immediately above first tarsal segment, removing both sex combs; 3, leg amputated immediately below first tarsal segment, sex combs not removed; 4, one sex comb removed by amputating one leg immediately above first tarsal segment, other leg left intact.)

Species and strain	Treatment	Mating period (h)	Females examined		
			Mated	Unmated	Total
D. mauritiana BG	1	24	103	7	110
	2	24	6	112	118
	3	24	105	15	120
	4	24	98	16	114
D. mauritiana mixed	2	24	21	106	127
	3	24	114	11	125
D. simulans Oxnard	2	24	113	13	126
	3	24	127	1	128
	2	6	64	61	125
	3	6	116	8	124
D. simulans Belmont	2	6	61	67	128
	3	6	118	8	126

Treatments (2) and (3) were then given to the Oxnard strain of *D. simulans*, again followed by a 24-h mating opportunity. Table 4 shows that although removal of sex combs significantly reduce the proportion of females inseminated ($G_1 = 13{\cdot}0$, $P < 0{\cdot}001$), a much higher percentage of females given treatment (3) were inseminated in *D. simulans* than in *D. mauritiana*. Because *D. simulans* appeared to mate more vigorously than *D. mauritiana*, treatments (2) and (3) were repeated, but with the mating opportunity reduced to 6 h. This reduction now reveals a very large absolute difference between treatments (2) and (3) in the proportion of *D. simulans* females inseminated ($G_1 = 61{\cdot}4$, $P < 0{\cdot}001$). Thus, as reported by Cook (1977), removal of sex combs delays but does not prevent copulation of *D. simulans* males. To determine if this apparent interspecific difference in mating vigour may only have been a difference between strains, treatments 2 and 3 were repeated on a strain of *D. simulans* from Belmont, Massachusetts. The results (Table 4) are virtually identical to those from the conspecific Oxnard strain.

These experiments show that the absence of the tarsal segment containing the

sex combs substantially reduces the mating ability of *D. mauritiana* males, but has a less serious effect on *D. simulans*. This loss of mating ability is not due to the operation itself, because amputation of the entire leg below the sex comb has almost no effect on the frequency of insemination. It is likely that the loss of mating ability is caused by loss of the sex comb itself, although it is a formal possibility that the first tarsal segment and not its associated sex comb is somehow responsible.

Table 5. *Abilities of* D. simulans *males with and without sex combs to grasp and mount females*

(Flies without combs correspond to treatment 2 in Table 4; those with combs to treatment 3 (both groups had some portion of the leg amputated). Eighteen replicate observation vials were made for each treatment (see text for further information).)

Treatment	Attempted grasps	Successful grasps (matings)
With combs	106	20
Without combs	192	5

Further insight into the function of this structure resulted from observation of males given treatments 2 and 3 in mating chambers with conspecific females. Males without sex combs had great difficulty grasping the female genitalia with their forelegs, a step that allows the male to hoist himself on to the female before intromission. To quantify this effect, two males from either treatments 2 or 3 were observed for 20 min in mating chambers with two virgin females. An 'attempted grasp' by a male was defined as a lunge with both forelegs at the extended female ovipositor, and a 'successful grasp' as an attempted grasp resulting in mounting and copulation. Table 5 shows that over the 20 min observation period, flies without sex combs (treatment 3) had a significantly lower proportion of grasps resulting in mating ($G_1 = 19{\cdot}1$, $P < 0{\cdot}001$) as well as a significantly lower proportion of matings ($G_1 = 9{\cdot}45$, $P < 0{\cdot}005$). Males without sex combs had great difficulty in grasping and mounting the female, confirming Cook's (1977) observation that sex combs are used in 'precision grasping' of the ovipositor.

Sex combs of both *D. simulans* and *D. mauritiana* were examined with a scanning electron microscope as a further investigation of the possible functions (chemosensory bristles in insects often have pores [Hodgson, 1974]). Plate IA shows the sex combs of *D. mauritiana*, which are structurally identical to those of *D. simulans*. The bristles have no pores, and are merely enlarged versions of setae present elsewhere on the body. An interesting feature of these combs is their association with projecting segments of exoskeleton which arise from the tarsus and appear to almost touch the base of each hair on the lateral side of the comb (Plate IB). If bent backwards, the sex comb bristles would touch these structures and possibly convey tactile information to the male. This further supports their function as grasping organs. Finally, inspection of the female ovipositor discloses a row of stiff bristles near the tip (Plate IC, arrow), which are curved in a way that would anchor the male's sex combs. Inspection of mating flies indeed showed that the sex combs contact this region during mounting.

It appears, then, that the primary function of sex combs in these species is to allow males a secure grasp on the extruded female genitalia before mounting. The significance of the *difference* in tooth number between *D. mauritiana* and *D. simulans* is more obscure, but it is notable that the former species, with a larger sex comb, suffers a more severe loss of mating ability when the structure is removed. It is possible that the increased size of this character is a selective response to other evolutionary changes that decreased the ability of *D. mauritiana* males to mate successfully.

(iii) *Genetic analysis of testis colour*

David *et al.* (1976) reported a colour difference between the testis of *D. mauritiana* and *D. simulans*: males of the former species have pale, yellow–white testes, while those of the latter species are a bright, buttery yellow. I confirmed this observation and found the colour difference most clearly developed in flies more than four days old. To investigate the genetic basis of this difference, I crossed the Oxnard strain of *D. simulans* with the Bowling Green strain of *D. mauritiana*. Virgin males of the two pure species and reciprocal hybrids between them were held five days at 23° and dissected in Ringer's solution. The colour of testes was classified by visual inspection as 'simulans type' (closer in colour to *D. simulans* testes than to *D. mauritiana* testes), 'mauritiana type' (vice versa) and 'intermediate' (not classifiable). Atrophied testes (see below) were not scored. This rather crude visual inspection was used because standard colour charts proved unsatisfactory, and because the method should be adequate to identify a difference resulting from a single allelic substitution if colours vary little within species but greatly between them. In all crosses, five-day-old males of both species were used as standards for classification.

Table 6 shows that the difference in colour between the species is almost diagnostic, as they overlap only slightly in colour (about 5 % of *D. mauritiana* males and 3 % of *D. simulans* males are 'intermediate'). Males from reciprocal F_1 interspecific hybrids (Table 7) have testes almost identical in colour to those of pure *D. simulans*, although when groups of testes from reciprocal F_1s are placed side by side, those with the *D. mauritiana X* chromosome are slightly lighter. Both F_1s are, however, well within the colour category of *D. simulans*, indicating dominance of whatever genes cause the colour difference.

Hybrid female offspring from the cross between *D. simulans* females and *D. mauritiana* males were backcrossed to both *D. simulans* and *D. mauritiana* males. Table 6 shows that, as expected, the backcross to *D. simulans* males gives testes identical in colour to *D. simulans* (dominance should also be operating here). Male offspring of the backcross to *D. mauritiana*, however, showed segregation of parental types. Although there is variation in testis colour among these offspring, the great majority fall within the range of *D. simulans* colour. The proportion of *D. mauritiana* 'types' in this class (0·130) is in fact statistically indistinguishable from the 7:1 $(1/2)^3$ or 0·125 proportion expected in a backcross involving the segregation of three recessive alleles that interact epistatically to yield the *D. mauritiana* type colour ($G_1 = 0{\cdot}10$, n.s.). There is certainly no evidence of the 1 : 1

ratio that would be expected if the character difference were caused by a substitution at a single locus, and the ratio also differs significantly from other plausible ratios such as 3:1 and 5:1.

Hybrid female offspring from this backcross to *D. mauritiana* were again backcrossed to *D. mauritiana* males. The assumption of three recessive loci (two on the autosomes and one on the sex chromosome) interacting epistatically to yield a *D. mauritiana* testis colour leads to an expectation in this cross of a 27/64 (0·42) proportion of *D. mauritiana*-type testes. The observed proportion of male offspring

Table 6. *Genetic analysis of testis colour in* D. mauritiana, D. simulans *and their hybrids.*

(Testes were classified as 'mauritiana-type' (closer to light yellow *D. mauritiana* testes), 'simulans type' (closer to deep yellow-orange *D. simulans* testes) or 'intermediate' (not closer in colour to one than the other). Crosses are designated by the female parent first, so that (S × M) × S represents offspring from backcross of hybrid females (themselves from a cross of *D. simulans* females to *D. mauritiana* males) to *D. simulans* males.)

Genotype	Simulans type	Mauritiana type	Intermediate	Total
D. simulans Oxnard	657	—	19	676
D. mauritiana Bowling Green	—	575	30	605
F_1 (M × S)	100	—	6	106
F_1 (S × M)	204	—	2	206
Backcross (S × M) × S	323	—	5	328
Backcross (M × S) × S	333	—	14	347
Backcross (S × M) × M	367	55	8	430
$Backcross_2$ [(S × M) × M] × M	327	169	23	519

with such testes (0·341) is in fact significantly smaller than this ratio ($G_1 = 13{\cdot}7$, $P < 0{\cdot}001$), but does not differ significantly from the 81/256 (0·32) proportion expected with the segregation of four recessive loci. Both backcrosses show that the colour difference is probably caused by at least three independent genetic factors (one on each autosome and one on the sex chromosome) that act epistatically. This is a minimum estimate, because in these backcrosses genes segregate in large blocks.

Ratios mimicking Mendelian segregation can sometimes be produced by the segregation of polygenes affecting a threshold character (Wright, 1968). One test of this possibility is to determine whether hybrid males with testes identical in colour to *D. mauritiana* (putative recessive homozygotes) breed true when crossed to pure *D. mauritiana* females. Fifty virgin males from the backcross [(S × M) × M] × M (see Table 7) were crossed to *D. mauritiana* females. The male parents were scored for testis colour after five days. Of the 48 males surviving, 16 had *D. mauritiana*-coloured testes. Only four of these produced offspring, as there is high sterility of backcross males (David *et al.* 1976). Three of these yielded progeny having only *D. mauritiana*- or intermediate-coloured testes, but one male produced among 31 offspring a single male with *D. simulans*-coloured testes. These results show that *D. mauritiana* colouration is probably the result of homozygosis for recessive, independently acting factors, but that there are additional complications of inheritance. The data indicate that the character has a polygenic basis

and is not the result of a single allelic substitution of large effect. Because of the pervasive linkage caused by three major chromosomes, three factors approaches the maximum number obtainable in these crosses, and it is likely that additional loci contribute to the interspecific difference in testis colour.

(iv.) *Developmental disharmony in species hybrids*

High levels of developmental anomalies have been reported in interspecific hybrids of *Drosophila melanogaster* and *D. simulans*, including abnormalities in chitinization of the abdomen, deviant sex ratios, gonadal atrophy and abnormal numbers (normally 8) of scutellar + dorsocentral bristles (Sturtevant, 1920, 1929; Biddle, 1943; Weisbrot, 1963). Interspecific crosses were made between *D. simulans* and *D. mauritiana* to determine whether similar amounts of disharmony existed in hybrids of the more closely related species. Such a finding would imply that most of the genic divergence leading to these anomalies may occur early in the process of speciation.

To establish the level of such anomalies *within* single species, I examined recently collected strains *D. simulans*, *D. mauritiana* and *D. melanogaster* as well as offspring of interstrain crosses. Backcrosses among geographic strains were also made for the first two species. All analyses scored at least 50 males and 50 females for each of six strains chosen to represent a variety of locations or six crosses among these strains. Anomalies were also measured in F_1 hybrids between *D. simulans* and *D. melanogaster*, and between *D. simulans* and *D. mauritiana*. Female hybrids of the latter two species were also backcrossed to both parental species. Because *D. simulans*/*D. melanogaster* hybrids are sterile, backcross hybrids can only be approximated by crossing genetically marked triploid females of *D. melanogaster* to irradiated *D. simulans* males. Data for a few hybrids of this type were kindly provided by Dr David Weisbrot (1963; pers. comm.). All interspecific hybrids except for these artificial backcrosses came from six crosses between different strains of the species. In all crosses, a sample of about 50 individuals of each sex was scored for the sum of dorsocentral + scutellar bristles and inspected for abnormal chitinization of the abdomen (Weisbrot, 1963).

As expected, the level of bristle abnormalities is low within each species and in interstrain crosses within each species (Table 7). *D. mauritiana* has a slightly higher frequency of bristle anomalies because of the presence of two strains with moderate frequencies of extra dorsocentral bristles. The results of the interspecific crosses are clear: the number of bristle anomalies is much higher in *D. simulans*/*D. melanogaster* hybrids than in *D. simulans*/*D. mauritiana* hybrids. The former cross gives 48 % of males with an abnormal number of bristles (mean 7·22) and 41 % of females (mean = 7·40). Hybrids between the latter species pair have abnormal bristles in only 0·2 % of males (mean = 8·00) and 1 % of females (mean = 8·01), a level similar to that of intraspecific crosses. The difference between the interspecific crosses in the number of anomalous individuals is highly significant for both males ($G_1 = 253{\cdot}2$, $P < 0{\cdot}001$) and females ($G_1 = 209{\cdot}0$, $P < 0{\cdot}001$). In the single 'artificial backcross' between *D. simulans* and *D. melanogaster* reported by Weisbrot (1963; pers. comm.), bristle anomalies are even more severe, while

Table 7. *Morphological abnormality in* D. simulans, D. melanogaster, D. mauritiana *and hybrids among them.*

(Six lines or six crosses were examined for each genotype.)

Genotype	Sex	*N*	Dorsocentral plus scutellar bristles: No. abnormal	Mean bristle no.	Standard error	Number with abnormal abdomen
			Pure species			
melanogaster	♂	330	1	8·00	0·003	0
	♀	326	9	8·01	0·012	1
simulans	♂	307	3	8·01	0·006	2
	♀	321	8	8·01	0·009	1
mauritiana	♂	314	8	8·03	0·009	2
	♀	312	32	8·10	0·024	14
			Interstrain crosses			
sim. × *sim.*						
F_1	♂	334	2	8·00	0·004	0
	♀	335	4	8·01	0·006	1
Backcross	♂	333	6	8·01	0·007	0
	♀	327	8	8·02	0·009	1
maur. × *maur.*						
F_1	♂	317	4	8·02	0·009	2
	♀	327	21	8·08	0·019	5
Backcross	♂	322	16	8·01	0·025	1
	♀	328	22	8·07	0·019	0
			F_1 hybrids			
mel. × *sim.*	♂	297	142	7·22	0·066	49
	♀	329	134	7·40	0·058	2
sim. × *maur.*	♂	336	1	8·00	0·003	1
	♀	407	6	8·01	0·005	0
			Backcross hybrids			
mel. × *sim.**	♂	10	6	2·50	0·428	—
	♀	5	4	5·80	0·663	—
sim. × *maur.*	♂	319	4	8·01	0·007	0
	♀	331	6	8·01	0·007	1

* Data from Weisbrot (1963 and pers. comm.).

D. simulans/*D. mauritiana* backcross hybrids again show a low level of anomalies not exceeding the background level within species (Table 7). The number of anomalous individuals again differs significantly between the two backcrosses (males: $G_1 = 33{\cdot}1$, $P < 0{\cdot}001$; females: $G_1 = 25{\cdot}0$, $P < 0{\cdot}001$).

Counts of abnormal abdomens give a similar result. All pure species, intrastrain crosses and interspecific crosses have a low level of such abnormalities except for male F_1 hybrids between *D. melanogaster* and *D. simulans*. The frequency of

abnormal abdomens in these hybrids significantly exceeds that of male *D. mauritiana*/*D. simulans* hybrids ($G_1 = 70{\cdot}1$, $P < 0{\cdot}001$). Although no measurement of such anomalies was made in artificial backcross hybrids between *D. simulans* and *D. melanogaster*, Weisbrot (1963; p. 1130) reported that they showed extreme phenotypic disturbances of the mouthparts, bristles, abdomens, genitalia, eye size and tarsi. No such anomaly was seen in either F_1 or backcross hybrids between *D. simulans* and *D. mauritiana*.

Another series of crosses was designed to measure the breakdown of developmental homeostasis in species hybrids by measuring the phenotypic difference among two characters that have very high genetic correlation: the number of bristles on the fourth and the fifth abdominal sternites. The genetic correlation between the two characters is 0·96 in *D. melanogaster* (Falconer, 1960), so that they appear to be influenced by the same genes. The difference between bristle counts on the two segments thus indicates the level of developmental aberration affecting the expression of identical genes in different parts of the body (Lewontin, 1956).

The intra-fly difference in bristle number was counted in 25–50 *D. melanogaster*/*D. simulans* and *D. simulans*/*D. mauritiana* F_1 hybrids from each of four hybrid crosses involving different geographical strains. Two intraspecific crosses between geographic strains of each of the three species served as controls.

The absolute average difference between segments for *melanogaster*/*simulans* hybrids was $2{\cdot}37 \pm 0{\cdot}35$ bristles for males (pooled mean and standard error), and $2{\cdot}16 \pm 0{\cdot}30$ for females. Asymmetry in *simulans*/*mauritiana* hybrids was $1{\cdot}34 \pm 0{\cdot}25$ bristles in males and $1{\cdot}38 \pm 0{\cdot}24$ in females; and the control intraspecific crosses had asymmetries of $1{\cdot}43 \pm 0{\cdot}22$ for males and $1{\cdot}51 \pm 0{\cdot}24$ for females. *t* tests of linear combinations of means from replicate crosses (Snedecor & Cochran, 1967) show that the *melanogaster*/*simulans* hybrids have significantly greater differences among segments than *mauritiana*/*simulans* hybrids (males: $t = 4{\cdot}48$, 242 D.F., $P < 0{\cdot}001$; females: $t = 3{\cdot}94$, 242 D.F., $P < 0{\cdot}001$). The former hybrids also differ significantly from interspecific controls (males: $t = 5{\cdot}65$, 242 D.F., $P < 0{\cdot}001$; females: $t = 3{\cdot}48$, 242 D.F., $P < 0{\cdot}001$). *Mauritiana*/*simulans* hybrids, on the other hand, do not differ significantly from controls (males: $t = 0{\cdot}45$, 242 D.F., $P > 0{\cdot}6$; females: $t = 0{\cdot}92$, 242 D.F., $P > 0{\cdot}3$). Again the older pair of species shows significantly higher levels of developmental breakdown than the younger pair.

Gonadal atrophy in males, another indication of developmental disharmony among *Drosophila* species (Pontecorvo, 1943*a*), was measured in offspring of *D. simulans* × *D. melanogaster* and *D. simulans* × *D. mauritiana* crosses. Data for testicular degeneration in artificial backcross hybrids of *D. simulans* and *D. melanogaster* was taken from Pontecorvo (1943*a*). Testes of four-day-old virgin males were dissected and inspected; completely withered, degenerate testes falling into category 'c' of Pontecorvo (1943*a*, p. 390) were scored as atrophied, any larger testes as non-atrophied. Table 8 shows that gonadal atrophy, like the previous characters, is more severe in *D. simulans*/*D. melanogaster* hybrids than in *D. simulans*/*D. mauritiana* hybrids; this is true for males in both the F_1 ($G_1 = 1206{\cdot}0$, $P < 0{\cdot}001$) and backcrosses ($G_1 = 58{\cdot}5$, $P < 0{\cdot}001$).

Sex ratios are extremely skewed in *D. simulans*/*D. melanogaster* hybrids: crosses

with *D. simulans* fathers produce virtually all females, the reciprocal cross virtually all males (Sturtevant, 1929). Six crosses between these species (Table 8) confirm these observations. Six F_1 crosses between *D. mauritiana* and *D. simulans* (Table 8), however, give sex ratios ranging from 0·46 to 0·52, with a mean male proportion of 0·48. These values are well within the normal sex ratios in crosses among conspecific strains (David *et al* 1974).

Table 8. *Testicular atrophy at 23° and sex ratio in* D. simulans × D. melanogaster *and* D. simulans × D. mauritian *hybrids.*

	Male gonads			Sex ratio		
Genotype	No crosses	*N*(total)	Fraction atrophied	No. crosses	*N*(total)	Mean proportion males
sim. × *mel.*						
F_1	4	333	1·00	6	856	0·99/0·00*
Backcross	1	65	0·66†	—	—	—
sim. × *maur.*						
F_1	9	883	0·04	6	2964	0·48
Backcross	7	1385	0·27	—	—	—

* 0·99 from crosses of *simulans* females × *melanogaster* males; 0·00 from the reciprocal cross.
† Data from Pontecorvo (1943*a*,*b*).

The studies of all five characters give consistent results: developmental disharmonies in *D. simulans*/*D. melanogaster* hybrids are more severe than in *D. simulans*/*D. mauritiana* hybrids. The lessened genetic divergence between the more recently derived species supports the theory that the genetic changes that produce aberrant hybrids occur gradually and are not concurrent with speciation.

(v). *Temperature-sensitive gonadal dysgenesis*

The similarity of testicular atrophy in male *D. simulans*/*D. melanogaster* hybrids to that occurring in hybrid dysgenic crosses of *D. melanogaster* led to the proposal that hybrid dysgenesis may be the cause of reproductive isolation between species (Kidwell & Novy, 1979; Rose & Doolittle, 1983; Ginzburg *et al.*, 1984). Although little testicular atrophy was found in *D. simulans*/*D. mauritiana* hybrids reared at 23° (see above), P–M dysgenic atrophy of both sexes in *D. melanogaster* is much enhanced by rearing at temperatures above 27° (Engels & Preston, 1979). The possibility of temperature-sensitive dysgenesis was therefore studied in *D. simulans*/*D. mauritiana* hybrids. Five interspecific crosses were made between various strains of *D. mauritiana* and *D. simulans*, and hybrids reared at low (23°) and high (30°) temperatures until adults eclosed. Male offspring were then collected as virgins, held for 4 days at 23°, dissected, and scored for testicular atrophy of the form described by Engels & Preston (1979), which is identical to the criterion of 'atrophied' gonads used above.

Atrophied testes were found in 31 of 558 hybrid males raised at 23° and in only 7 of the 477 males raised at 30°. This difference is statistically significant but in

the wrong direction. The degree of atrophy in both crosses is significantly less than the proportion of sterile males that have uni- or bilateral testicular degeneration in GD dysgenic crosses (0·43–0·84 [Engels & Preston, 1979; Thompson, Henderson & Woodruff, 1980]).

Temperature-sensitive sterility of hybrid females was also examined in two interspecific crosses involving two strains of both *D. mauritiana* and *D. simulans*. For each cross, 50 females were reared at both temperatures. After eclosion, each female was individually mated to two males (one from each species), and vials scored for larvae after 7 days. Of the 100 females raised at 23°, 99 were fertile, and one produced no offspring but had normal ovaries upon dissection. Of females raised at 30°, 98 produced progeny, one died and one produced no progeny but had normal ovaries. Further dissection of 100 females from the two 30° crosses showed all with normal-sized ovaries, with no evidence of the bilateral or unilateral ovarian atrophy found in virtually every female reared at high temperature in P–M dysgenic crosses (Engels & Preston, 1979; Schaefer *et al.* 1979).

These crosses, then, provide no evidence for gonadal atrophy, temperature-sensitive or otherwise, in F_1 hybrids of *D. simulans* and *D. mauritiana*. The sterility of male *D. simulans*/*D. mauritiana* hybrids has little to do with gonadal atrophy but results instead from failed spermatogenesis (Coyne, 1984).

3. DISCUSSION

(i) *Age of the species*

The reproductive, electrophoretic and chromosomal relationships among these species show that *D. mauritiana* diverged from *D. simulans* much more recently than from *D. melanogaster*. This conclusion is completely confirmed by DNA sequencing, generally considered the most accurate biochemical estimator of relative divergence times. Bodmer & Ashburner (1984) used genetic divergence at the third codon position of the alcohol dehydrogenase locus to place the divergence of *D. mauritiana* from *D. simulans* at 2·9 million years ago, and that of the *D. simulans*/*D. mauritiana* ancestor from *D. melanogaster* at 3·9 million years ago. Cohn *et al.* (1984) used total DNA sequence at the same locus to place the former divergence at 2·7 million years ago and the latter at 4·7 million years, and estimated respective divergence times of 0·4 and 0·8 million years from electrophoretic differences. These estimates are probably erroneous because they are calibrated from mammalian data, but they show that the relative ages of the speciation events differ by a factor between 1·4 and 2. Coupled with the biogeography of these species, these data make the *D. simulans*/*D. mauritiana* divergence one of the most well-documented founder events in the genus, and justify a comparison of this speciation event with the more remote one separating *D. simulans* and *D. melanogaster*.

(ii) *Genetic basis of species differences*

Templeton (1981, 1982) divides the genetic basis of species differences into three categories: type I, comprising differences caused by many genes of small effect; type II, including differences caused by one or a few major genes and several modifiers, and type III, including differences due to one or two loci of large effect. He suggests that speciation accompanying genetic revolutions would favour types II and III, while allopatric speciation caused by gradual adaptive divergence would favour type I. Wright (1982) also proposed that type III monogenic architectures would be associated with relaxed or strong selection in a novel environment, as might occur during island colonization, a notion also supported by advocates of the theory of punctuated equilibrium (Stanley, 1979; Gould, 1980).

This study brings to four the number of genetically analysed differences between *D. simulans* and *D. mauritiana*. Although these analyses are limited by the paucity of genetic markers existing in these species, they give enough evidence to reject the idea that single genes of large effect have been important in this speciation event. The difference in genital morphology, due to at least five loci, has a genetic architecture of either type I or type II (Coyne, 1983); the genetic divergence contributing to hybrid male sterility, attributable to at least six loci, has a type I or type II architecture (Coyne, 1984, 1985); the difference in sex comb tooth number (at least five loci) has type I architecture; and the difference in testis colour is due to at least three epistatically acting genes. All of these studies revealed the largest number of genetic differences permitted by the method of analysis, so all are minimum estimates of gene number.

It would be foolish to generalize about the genetics of species differences based on studies of only one species pair. Nevertheless, a body of studies similar to this constitutes a strong test of speciation theory. Combining this study with work on other animal species (summarized in Coyne, 1983), one finds no evidence for the importance of macromutations in speciation.

(ii) *The evidence for genetic 'revolutions'*

The genetic pattern of species differences has been offered as a means of discriminating between Darwinian speciation and speciation involving genetic drift, because differences involving one or several loci of large effect have been said to be more frequent results of genetic revolutions (Templeton, 1981, 1982). This appears doubtful, for the strong selection accompanying entry of a new ecological niche can also fix genes with large phenotypic effects by a purely Darwinian process of adaptive divergence (Lande, 1983; Wright, 1982). In addition, recent theory shows that genetic differences based on many substitutions of small effect may actually be the most probable outcome of genetic revolutions (Barton & Charlesworth, 1982). Thus, although the discovery of occasional macromutational differences between species implicates strong selection during their divergence, it allows no discrimination between purely selective processes and those involving a combination of selection and drift. This problem is exacerbated because even those

who advocate genetic revolutions believe in their extreme rarity (Templeton, 1980; Mayr, 1982).

It is in fact almost impossible to find genetic evidence that genetic revolutions have caused speciation. The most explicit models of this process propose that the interaction of selection and genetic drift propels species across valleys of lowered fitness to new adaptive peaks (Carson & Templeton, 1984). This model is similar to Wright's (1932, 1982) shifting balance theory of evolution, except that the peak shift during genetic revolutions causes reproductive isolation and involves only one deme instead of many. There is, however, no mathematical model showing that founder effects can lead with reasonable probability to rapid and complete reproductive isolation of populations. Some support for this possibility would be given by evidence that the development of reproductive isolation involves evolutionary changes that at some point lower the mean fitness of populations. This conclusion would require fairly complete knowledge of the frequencies, fitnesses and interactions of alleles causing reproductive isolation. Needless to say, we have no such knowledge.

A weaker approach would be a search for any evidence that genetic drift occurred during speciation. The fixation of chromosome inversions which have low fitness when heterozygous implies genetic drift. Such fixations are often observed among related species (White, 1978; chapter 3). It has been noted, however, that these rearrangements may not cause complete reproductive isolation and may be a correlate instead of a cause of speciation (Charlesworth *et al.* 1982; Spirito, Rossi & Rizzoni, 1983). In addition, many species that have experienced founder events, such as *D. mauritiana* and *D. sechellia*, are identical in chromosome sequence to their putative ancestor (Lemeunier & Ashburner, 1976; 1984). It is also impossible to determine whether observed fixations of rearrangements among species occurred during or after speciation. Finally, there is at least one model (Nei, Maruyama & Wu, 1983) showing that genetic drift can enhance the rate of speciation in ways that do not lower population fitness, so that the demonstration of drift becomes necessary but not sufficient evidence for genetic revolutions.

It is clear that no observation of morphological or behavioural differences among populations supports genetic revolutions, because there is always an alternative explanation involving a combination of natural selection and novel environment. Thus, although the speciose Hawaiian *Drosophila* are frequently offered as examples of genetic revolutions via founder-event speciation (and appear to have inspired many of these theories), there seems to be no evidence that genetic drift has had a hand in the morphological and reproductive differences among species. Hawaiian *Drosophila* do not differ by fixed chromosome rearrangements that would result from genetic drift (Carson & Kanershiro, 1976), nor do they have low allozyme heterozygosity (Templeton, 1980). Aside from frequent sexual dimorphism, the radiation of this group does not differ from other adaptive radiations that are widely accepted as resulting from natural selection in new habitats; and it is puzzling that Hawaiian *Drosophila* are regarded as products of genetic revolutions.

In light of the facts that (1) there is no theoretical model showing that founder events can lead to rapid and complete reproductive isolation based on a few loci,

(2) there is no genetic or observational evidence that speciation events involve genetic drift, and (3) the conventional explanations for both speciation and adaptive radiation have strong theoretical, experimental and natural-historical support, it seems premature to reject the neo-Darwinian model for adaptive radiations on islands.

(iv) *The accumulation of genetic divergence during speciation*

This study clearly rejects the hypothesis that older and younger species pairs in the *D. melanogaster* group show equivalent amounts of genetic divergence resulting in abnormal morphology of species hybrids. All measures of developmental anomaly are clearly more advanced in hybrids between the more distantly related species pair. Only in male sterility of F_1 hybrids do the two species pairs show equal degrees of abnormality, and even in this case hybrids between the older pair have more severe gonadal atrophy. Female sterility is, of course, complete in hybrids between the older species pair and non-existent in hybrids between the younger.

The correlation between divergence time and degree of morphological disturbance in hybrids supports the idea that the attainment of reproductive isolation is only one step – albeit an an important one – in a continuous process of genetic differentiation among isolated populations. Douglas & Avise (1982) also supported this idea by finding that fish lineages with higher rates of speciation showed no increase in morphological diversification. There is no experimental support for the idea that most interspecific divergence in morphology and development is concurrent with the attainment of reproductive isolation.

Based as it is on three species, this conclusion clearly requires confirmation from a more extensive taxonomic group. The other species of the *D. melanogaster* subgroup are ideal subjects for such work. Although their interfertility is still not completely known, their phylogeny and relative divergence times have been determined by DNA sequencing (Bodmer & Ashburner, 1984).

(v) *Transposable elements and speciation*

There is no evidence in *D. simulans*/*D. mauritiana* hybrids for the type of gonadal atrophy ('GD sterility') caused by the P–M system of hybrid dysgenesis in *D. melanogaster*, nor is the sterility of the F_1 hybrids attributable to gonadal atrophy.

Much attention has been lavished on the possibility of dysgenesis-induced speciation, but the only supporting evidence appears to be the observation that species hybrids (particularly between *D. melanogaster* and *D. simulans*) often have atrophied gonads similar to those resulting from hybrid dysgenesis in *D. melanogaster* (Bregliano & Kidwell, 1983). More striking, however, are the *differences* between dysgenic sterility and interspecific hybrid sterility. Dysgenic sterility is normally found only at high temperatures, while interspecific sterility occurs at normal rearing temperatures. In addition, both I–R and P–M dysgenesis in *D. melanogaster* have more severe effects in females, while hybrid sterility in *Drosophila* is far more common in males (Kidwell *et al.* 1977; Bock, 1984). Another form of dysgenic sterility ('SF sterility'), results from the I–R dysgenic system.

SF females lay fertilized eggs that fail to develop past the first few cleavages (Bregliano & Kidwell, 1983). This form of sterility is certainly absent in *D. simulans*/*D. mauritiana* hybrids (all females are fertile) and fails to explain widespread male-limited sterility in *Drosophila* hybrids.

A more convincing implication of hybrid dysgenesis in speciation would require demonstrating (1) differences between closely related species in the composition or number of transposable genetic elements or in the cytotype conferring sensitivity to them and (2) that these differences are responsible for reproductive isolation. Increased male recombination, elevated mutation rates, and temperature-sensitive gonadal atrophy are not, of course, bases for reproductive isolation. It is therefore premature to conclude that 'transposable elements may be the central biological agent controlling much of the evolutionary process in sexually reproducing organisms including the generation of mutational diversity and reproductively isolated lines of descent involved in speciation' (Ginzburg *et al.* 1984; p. 339).

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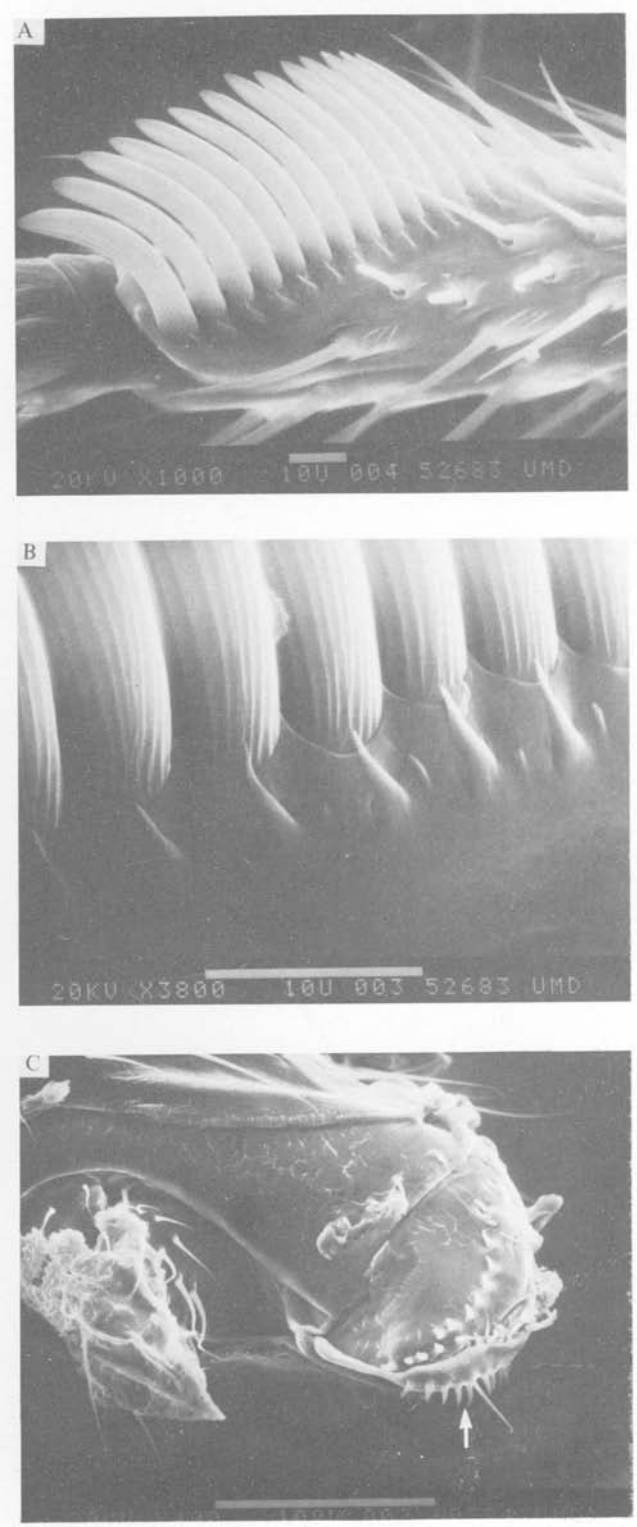
EXPLANATION OF PLATE

Scanning electron micrographs of *D. mauritiana* adults.
(A) Male sex comb on the first tarsal segment. Grooved setae differ in size but not appearance from bristles elsewhere on the body (length of line = 10 μm).
(B) Closer view of the base of sex comb setae. Note structures arising from the leg exoskeleton that nearly touch the base of each bristle (length of line = 10 μm).
(C) Female ovipositor. Note row of recurved hairs (arrow) on either side of the genital opening. Male sex combs appear to touch this region when the female is mounted (length of line = 100 μm).



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