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Sexual isolation in *Drosophila melanogaster*: A possible case of incipient speciation

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ABSTRACT **It is generally believed that *Drosophila melanogaster* has no closely related species with which it can produce the viable and fertile hybrids that are essential for the genetic analysis of speciation. Following the recent report of molecular differentiation between a Zimbabwe, Africa, population and two United States populations, we provide evidence that strong sexual isolation exists between the *D. melanogaster* population in Zimbabwe and populations of other continents. In the presence of males of their own kind, females from most isofemale lines of Zimbabwe would not mate with males from elsewhere; the reciprocal mating is also significantly reduced, but to a lesser degree. The genes for sexual behaviors are apparently polymorphic in Zimbabwe and postmating reproductive isolation between this and other populations has not yet evolved. Whole chromosome substitutions indicate significant genetic contributions to male mating success by both major autosomes, whereas the X chromosome effect is too weak to measure. In addition, the relative mating success between hybrid and pure line males supports the interpretation of strong female choice. These observations suggest that we are seeing the early stages of speciation in this group and that it is driven by sexual selection. The genetic and molecular tractability of *D. melanogaster* offers great promise for the detailed analysis of this apparent case of incipient speciation.**

The difficulties in studying the genetics of speciation can arise from several sources. First, the species in question may have diverged beyond the incipient stage. Many genetic differences between them could have accumulated after speciation had been completed and the information on the population genetic dynamics of speciation may have been lost. Ideally, we would like to observe variation in genes of reproductive isolation that are still in the process of becoming fixed. Polymorphisms of such genes within species would suggest speciation in *flagrante delicto* (1). The second difficulty arises when the species of interest does not lend itself to extensive and detailed genetic analysis. The conspicuous absence of a species that could hybridize with *Drosophila melanogaster* to produce fertile progeny is the prime example of the second point. To some degree, all studies of the genetics of postmating reproductive isolation encounter the two problems (2). Genetic analysis of premating sexual isolation also confronts a third difficulty in that mating behaviors are often labile (3–8). Finding a system of sexual isolation associated with robust behavioral phenotypes is thus crucial for genetic studies of premating isolation.

Recently, a collection of isofemale lines of *D. melanogaster* from Zimbabwe, Africa, was reported to show a surprisingly high level of DNA sequence divergence at several nuclear genes compared to flies from North American populations (9). In light of previous observations that flies of this species collected over a wide geographical range are very similar in their nuclear DNA polymorphisms (10, 11), the observations suggested much more local population differentiation in Africa than had been hinted in the literature (12–14). Since there is no record of postmating reproductive isolation and a large-scale survey found little sexual isolation between world-wide samples of *D. melanogaster* (15), we were interested in whether any reproductive isolation is associated with the population differentiation seen in Africa. In this report, we present evidence that the Zimbabwe population reported in ref. 9 is strongly sexually isolated from other African populations as well as all *D. melanogaster* strains from other continents examined so far.

MATERIALS AND METHODS

Fly Strains. The Zimbabwe isofemale lines (designated Z) were collected in 1990 from Sengwa Wildlife Reserve (9). Nine were used initially (Z29, -30, -34, -53, -56, -2, -5, -6, and -10) but we subsequently concentrate on three of them (Z30, -29, and -53). For the common type *D. melanogaster* lines (designated C), we often use one isofemale line from France (FrV3-1) and another from California (Highgrove, HG). Other C lines used are OK17 (Botswana), LA69 (Northern Zambia), Closs 23 (New York), BL-10 (Australia), Arv-4 (California), and Vin-9 (Ontario, Canada). All of these are isofemale lines established in the 1980s. Isochromosomal II lines from Malawi (MW-33 and -44) and Ivory Coast (LM-15 and -25) were described in Benassi *et al.* (16).

No Choice Experiments. All mating experiments were done at "dawn" (around the time the daily light cycle starts) and at room temperature. Flies were 3–4 days old at the time of mating. Five virgin females and males of the designated type were placed in a clean vial. Copulations were recorded at 5- to 10-min intervals for an hour and then the flies were transferred to a vial with food. After 1 day, each female was separated into a single vial for progeny detection. We dissected many of the females that failed to produce larvae after a week for the presence of sperm in their seminal receptacles to confirm that they were uninseminated, rather than sterile. Experiments of Tables 1, set a, and 2–4 were done in the Wu laboratory at the University of Chicago and experiments of Table 1, sets b and c, were done in the Aquadro laboratory at Cornell University. For brevity, we always write A × B to indicate A females × B males. The G statistics is used to calculate the level of significance (17).

Multiple Choice Experiments. All experiments were also done at dawn. Flies were 3–7 days old at the time of mating (unless specified). Virgin females and males of two tested

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Abbreviation: DI, discrimination index.

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strains were simultaneously released into a population cage of about 0.034 m^3 in volume made of Plexiglas. Usually, 55–60 flies of each sex of each genotype are used (thus, 220–240 flies in a cage). Flies of one strain were fed green-colored food and flies of the other were fed red-colored food 12–24 hr prior to the experiment. We normally use one drop of the food coloring on the surface of the medium. Each copulating pair was aspirated out of the cage, placed on a CO_2 stage to determine their coloration, and then returned to the cage. Ambiguity in type determination is very rare (<1%) if the flies were deprived of colored food for no more than 3 hr. Occasional uncertainty was confirmed by dissecting out the gut. Within 1.5 hr, usually 70–100% of C-type females copulate (variable for Z-type females, depending on the males' genotypes).

Discrimination Index (DI). Let the observed numbers of mating between strain A and B be: n_{AA} (A females × A males), n_{AB} (A × B), n_{BA} (B × A), and n_{BB} (B × B). We use a (relative) DI defined as $DI = -\ln(n_{AB}n_{BA}/n_{AA}n_{BB})$. DI = 0 indicates no difference in mating preference between strain A and B and a positive value means homogamic matings are more frequent than expected. The variance, obtained by the delta method, is $Var(DI) = 1/n_{AA} + 1/n_{BA} + 1/n_{BA} + 1/n_{BB}$, which was first given by Fisher and rederived by Maruyama and Crow (18) in their viability study. We chose DI over other indices (19) because our unpublished results have shown that the DI value between strain A and B, DI(AB), is usually close to DI(AH) + DI(HB), where H is the hybrid between A and B. This additivity is convenient in our subsequent genetic analysis of the relative mating success of males of various introgression genotypes (H.H. and C.-I.W., unpublished results).

RESULTS

Preliminary Observations. Four Z lines were tested against five C lines in a no choice experiment, as presented in Table 1, set a. (All test statistics are given in the legends.) It is clear that Z females do not mate readily with C males within 1 hr or within 1 day, compared with other crosses. Moreover, there is a slight indication that C female by Z male matings also proceeded more slowly than within-type matings. The experiment was repeated with additional lines, as shown in Table 1, set b. The results are in general agreement except that Z × Z crosses proceeded significantly more slowly in set b. The difference may be due to the fact that the measure (% mated in a fixed period of time) is sensitive to strains used as well as slight variations in experimental conditions (e.g., lighting). Indeed, the proportion of Z30 and Z53 females mated in 1½ hr in seven multiple choice experiments varies greatly, ranging from 38% to 86% with a mean of 60% (see the next section). For this reason, we have switched to the multiple choice experiments, which measure *relative* mating success.

Table 1. Preliminary observations in no choice experiments

♀ × ♂	% mating	
	In 1 hr	In 1 day
	Set a	
1. Z × Z	60 (68/114)	93 (54/58)
2. Z × C	2.9 (9/312)	22 (30/134)
3. C × Z	38 (53/141)	98 (122/124)
4. C × C	57 (40/70)	98 (54/55)
Set b (replicates of crosses of set a)		
5. Z × Z	29 (73/249)	76 (187/247)
6. Z × C	8.8 (20/226)	30 (67/221)
7. C × Z	34 (84/246)	95 (227/238)
8. C × C	53 (129/245)	99 (239/241)
	Set c	
9. Z′× Z′	60 (47/78)	95 (73/77)
10. Z′× C	42 (30/71)	97 (69/71)
11. C × Z′	57 (44/77)	100 (77/77)
12. C × C	57 (40/70)	100 (70/70)

Four Zimbabwe isofemale lines (Z30, -34, -53, and -56) were used in set a. For the C lines, the standard Canton-S stock and four other *D. melanogaster* isofemale lines were used: one each from California (Highgrove), New York (Closs 23), France (FrV3-1), and Australia (BL-10), respectively. For set b, Z strains included the four lines above and Z2, -5, and -10. C strains were represented by a line from California (Arv-4) and another from Quebec (Vin-9), also used in set c. The Z′ strains in set c are Z6 and Z29. Below are the G test results between crosses. $P < 0.01$: cross 1 vs. 2 (1 hr and 1 day), cross 3 vs. 4 (1 hr), cross 5 vs. 6 (1 hr and 1 day), cross 7 vs. 8 (1 hr). $P < 0.05$: cross 9 vs. 10 (1 hr). All other comparisons between the adjacent crosses (11 vs. 12, 9 vs. 10, etc.) are not significant.

It became apparent that, among the Zimbabwe lines used, two somewhat discrete types of behaviors could be discerned. Seven of the nine lines exhibit the behavior presented in Table 1, sets a and b, whereas two of them exhibit the behavior of Table 1, set c. Females of these two lines (Z6 and Z29) mated more frequently with C males than did other Z females ($P << 0.01$ for comparisons between cross 10 vs. 2 and cross 10 vs. 6 of Table 1), although these females still mated with their own kind more readily than with C males ($P < 0.05$ for cross 9 vs. 10). We will present the mating behavior of each line separately in the next section on multiple choice experiments.

Multiple Choice Experiments. Briefly, 50–60 virgin females and males from each of the two tested lines were released into a population cage and copulating pairs were aspirated out for type determination (see *Materials and Methods*). There is no effect of food coloring on mating as reversing coloring between successive experiments yielded the same results; for example, among the seven Z × C crosses of Table 2, Z lines were fed the green dye in three crosses and the red dye in the other four crosses. We also ran two control experiments by feeding flies of the same strain different dyes. The DI was −0.43 ± 0.67 for FrV3-1 and −1.34 ± 0.72 for Z53, respectively. Since most observations of Tables 2 and 4 show significant positive DI values (favoring homogamic mating), the observed differences could not be due to food coloring.

A positive DI value indicates more homogamic mating than expected. We shall use DI of 3 as indicating strong premating isolation. DI ≥ 3 is equivalent to $n_{AB}n_{BA}/n_{AA}n_{BB} < 0.05$. (A high DI value can be explained by either female or male preference but our interpretation favors female preference primarily because of additional information; see *Discussion.*) Thus, crosses 1–7 of Table 2 all suggest that Z females' acceptance of C males over Z males (n_{AB}/n_{AA}) is <5% of C females' relative acceptance for the same two types of males (n_{BB}/n_{BA}). All seven DI values are highly significantly different from 0. In all cases, $n_{AA} >> n_{AB}$ and $n_{BB} > n_{BA}$, suggesting that sexual isolation is bidirectional, very strong between Z females and C males and weaker (but still substantial) in the reciprocal direction.

We show in Table 1 that two (Z29 and Z6) of the nine Z lines exhibited a relatively high level of Z × C mating. Table 2, set b, shows that Z29 is in fact "intermediate" between Z and C and, hence, is referred to as Z′. Although Z29 females mated with C males at an appreciable rate (DI between 1 and 2), Z29 males also succeeded in mating with Z53 or Z30 females with some regularity. In contrast, an inspection of crosses 3–7 indicates that Z53 females never mated with C males in >180 copulations. Clearly, Z29 line is not of the C type as might have been suggested by the female behavior.

Another line of interest in the non-Zimbabwe African sample is LA69 from Northern Zambia, which shows intermediate behavior between Z53/Z30 and Z29. LA69 females mated rarely with HG or FR males but LA69 males mated frequently with Z53 females. Note that LA69 males perform poorly against Z53 males with respect to both types of females. We classify LA69 as Z′ based on the females' willingness to accept some HG males (cross 12). There is in fact a continuum

Table 2. Multiple choice experiments

A (♀A, ♂A)	B (♀B, ♂B)	(n_{AA}, n_{AB}, n_{BA}, n_{BB})	DI*
	Set a: Z × C		
1. Z30 (66, 63)	FR (67, 62)	(38, 0, 16, 47)	∞ (5.41 ± 1.45)
2. Z30 (53, 51)	HG (56, 57)	(26, 2, 9, 49)	4.26 ± 0.82
3. Z53 (56, 50)	FR (60, 55)	(27, 0, 22, 18)	∞ (3.79 ± 1.46)
4. Z53 (55, 36)	HG (49, 30)	(21, 0, 12, 30)	∞ (4.65 ± 1.47)
			(4.78 ± 1.64)†
5. Z53 (55, 55)	LM (62, 60)	(41, 0, 17, 33)	∞ (5.07 ± 1.45)
6. Z53 (57, 60)	MW (63, 58)	(49, 0, 9, 32)	∞ (5.85 ± 1.47)
7. Z53 (60, 63)	OK (63, 59)	(46, 0, 15, 37)	∞ (5.42 ± 1.45)
	Set b: Z′ × C or Z × Z′		
8. Z29 (38, 32)	HG (49, 38)	(11, 7, 11, 20)	1.05 ± 0.61
9. Z29 (55, 60)	LM (59, 69)	(38, 12, 17, 34)	1.85 ± 0.44
10. Z53 (64, 43)	Z29 (55, 48)	(25, 8, 22, 24)	1.22 ± 0.50
11. Z30 (54, 59)	Z29 (77, 46)	(22, 4, 24, 33)	2.02 ± 0.61
12. LA69 (49, 63)	HG (55, 62)	(17, 3, 31, 34)	1.83 ± 0.67
13. LA69 (66, 57)	FR (52, 56)	(31, 1, 13, 34)	4.40 ± 1.07
14. Z53 (60, 60)	LA69 (59, 67)	(27, 9, 23, 12)	0.45 ± 0.52
	Set c: Z × Z or C × C		
15. Z53 (64, 57)	Z30 (63, 52)	(26, 26, 25, 9)	−1.02 ± 0.48
16. Z53 (67, 57)	Z56 (65, 57)	(24, 23, 18, 11)	−0.45 ± 0.48
17. HG (68, 68)	FR (59, 60)	(34, 12, 25, 27)	1.11 ± 0.44
18. HG (60, 60)	LM (60, 60)	(37, 16, 23, 19)	0.65 ± 0.43
19. HG (59, 63)	MW (67, 59)	(32, 26, 17, 26)	0.63 ± 0.41
	Set d: *D. melanogaster* × *Drosophila simulans*		
20. HG (57, 56)	LA4 (60, 57)	(48, 0, 0, 20)	∞ (9.64 ± 2.84)
21. Z30 (59, 53)	LA33 (73, 55)	(38, 0, 0, 51)	∞ (11.0 ± 2.84)

Strain A is given before strain B. ♀A and ♂A are the numbers of females and males of strain A used in the experiment; ♀B and ♂B are the numbers of females and males of strain B. The observed numbers of matings are n_{AA} (A ♀ × A ♂), n_{AB} (A × B), n_{BA} (B × A), and n_{BB} (B × B). DI = $-\ln(n_{AB}n_{BA}/n_{AA}n_{BB})$. When DI = ∞ (because $n_{AB} = 0$), we calculate the new DI ± SE by making $n_{AB} = 0.5$. These estimates of DI are enclosed in parentheses.

*Mean ± SE.

†The value is obtained by summing up the three intervals Z53 vs. Z/H, Z/H vs. H/Z, and H/Z vs. HG.

of behavior. The classification of Z (vs. Z′) is artificially based on the DI value being >3.0 over several different crosses.

Sexual isolation within the Z type and C type is presented in Table 2, set c. The DI value varies between −1 and +1 and in only one of the five cases (cross 17) is homogamic mating significantly more common than expected. Complete bidirectional sexual isolation is observed between *D. melanogaster* and *D. simulans* (Table 2, set d), as expected.

Genetics and Mating Behavior. To address the robustness of the observed sexual isolation, we have carried out multiple choice experiments under a range of environmental and physiological conditions—age, crowding, temperature, light, and a different set of experimental conditions. Sexual isolation between Z females and C males is indeed very robust against such variations (unpublished results). So far, genetic manipulation remains the only way to change the mating behavior of the Zimbabwe flies. In this report, we present mating preferences of F_1 and F_2 flies in Tables 3 and 4. A detailed genetic analysis will be given elsewhere (H.H., C.-I.W., and M.-L.W., unpublished results).

Comparing crosses 1 and 2 of Table 3 with crosses 1 and 2 of Table 1, we find F_1 males' performance to be intermediate between the two parental species. Comparisons between cross 1 and cross 3 ($P < 0.01$) and between cross 2 and cross 4 ($P < 0.02$) of Table 3 also corroborate the interpretation that Z females (but not C females) do not mate well with males bearing C autosomes. Since males in cross 1 of Table 3 do not mate significantly better than males in cross 2 ($P > 0.1$), the contribution of the X chromosome to the "Zimbabwe maleness" is probably limited. The slight difference may not be due to the greater "Zimbabweness" in males of cross 1 because the same males are also more successful with C females ($P > 0.05$ for cross 3 vs. 4). F_1 females accepted C males much less readily than they accepted Z males ($P < 0.01$ for cross 5 vs. 6).

A greater resolution of the mating preferences of F_1 hybrids can be obtained by substituting hybrids in the multiple choice experiments as shown in Table 4. In this table, H/Z stands for F_1 hybrids from the cross of HG females to Z53 males (H/Z males = X/Y; $+^z/+$; $+^z/+$), whereas Z/H are F_1 hybrids from the reciprocal cross (Z/H males = X^z/Y; $+^z/+$; $+^z/+$). The comparison is between each pair of the adjacent types in the sequence HG – H/Z – Z/H – Z, which increases in the Zimbabwe content from left to right (crosses 1–4). Crosses 1 and 2 are identical except that the Z females were from different lines.

In crosses 1 and 2 of Table 4, it is shown that H/Z males are favored by Z females over HG males very strongly. Note that a DI > 3.0 indicates Z females' preference (relative to that of HG females) for HG males is <5% of its preference for H/Z males. In other words, one copy of each of the two major autosomes from Zimbabwe is sufficient to raise the males' chance of being accepted by Z females by >20-fold. Nevertheless, H/Z males were not able to monopolize the mating with Z females as did Z males. Apparently, Z53 females' unwillingness to mate with HG males is not absolute—in the absence of their own kind, these females would settle for the less desired. Although Z females are highly discriminatory, their preference for Z/H males over H/Z males, relative to that of HG females, is too weak to measure (cross 3). The results confirm our earlier suggestion that the X chromosome has no detectable effect on the Zimbabwe maleness.

Because H/Z (and presumably Z/H) males are favored strongly by Z females over HG males, one might expect these F_1 hybrid males to be sufficiently Zimbabwe-like that they could compete well with Z males. Cross 4 shows that this is not

Table 3. No choice experiments on hybrids

♀ × ♂	% mating In 1 hr	% mating In 1 day
Set a: F_1 flies		
1. Z × X^z; $+^z/+$; $+^z/+$	32 (16/50)	81 (35/43)
2. Z × X; $+^z/+$; $+^z/+$	24 (12/49)	76 (31/41)
3. C × X^z; $+^z/+$; $+^z/+$	66 (33/50)	—
4. C × X; $+^z/+$; $+^z/+$	48 (24/50)	—
5. F_1 × Z	68 (39/57)	—
6. F_1 × C	39 (16/41)	84 (31/37)
Set b: F_2 males with whole chromosome substitutions		
7. Z × X/Y; *bw/bw*; *st/st*	0 (0/75)	37 (22/60)
8. Z × X^z/Y; *bw/bw*; *st/st*	1.1 (1/94)	37 (30/82)
9. C × X^z/Y; *bw/bw*; *st/st*	10 (2/20)	100 (20/20)
10. Z × X/Y; *+/bw*; *st/st*	5.6 (3/54)	30 (16/53)
11. Z × X^z/Y; $+^z$/*bw*; *st/st*	29 (25/87)	61 (48/79)
12. Z × X/Y; *bw/bw*; *+/st*	9.1 (3/33)	53 (17/32)
13. Z × X^z/Y; *bw/bw*; $+^z$/*st*	33 (31/94)	83 (73/88)
14. Z × X/Y; *+/bw*; *+/st*	24 (10/42)	73 (29/40)
15. Z × X^z/Y; $+^z$/*bw*; $+^z$/*st*	65 (48/74)	87 (62/71)

F_1 hybrids were derived from cross 2 or 3 of Table 1, set a. F_1 males from the two reciprocal crosses are different as indicated by their proper X chromosome designation. Data not collected are indicated by a dash. F_2 males in crosses 7–15 were derived from the crosses below. Parental generation: C(1)DX*yf*/Y; *bw/bw*; *st/st* × X/Y; *+/+*; *+/+* (or X^z/Y^z; $+^z/+^z$; $+^z/+^z$), where the females bear the attached X chromosomes, C(1)DX*yf*, and are homozygous for the *bw* (brown) and *st* (scarlet) mutation on the second and third chromosome, respectively. Males used were either from each of five Z lines (wild type with superscript z) or from any of the three C lines (HG, FrV3-1, and BL-10). F_1 males were then backcrossed to C(1)DX*yf*/Y; *bw/bw*; *st/st* virgin females. In F_2, males are either white-eyed (X/Y; *bw/bw*; *st/st*), scarlet-eyed (X/Y; *+/bw*; *st/st*), brown-eyed (X/Y; *bw/bw*; *+/st*), or wild type (X/Y; *+/bw*; *+/st*). Because there is no recombination in males, these are whole chromosome substitutions. The experiments, starting from the parental generation, were repeated once and the results are highly consistent.

true. Z males' success in mating with Z females is still 5-fold higher ($\approx 34 \times 29/[12 \times 17]$) than Z/H males'. Thus, the hybrids' relative success in mating with Z females can vary nearly 100-fold (from <5% to 500% of their competitors), depending on the genotype of other males. The observation strongly implicates female choice as the main cause of discrimination. We may sum up the DI values of Z53 vs. Z/H, Z/H vs. H/Z, and H/Z vs. HG of Table 4 to obtain a value of 4.8 ± 1.64 for Z53 vs. HG. This suggests that the probability of Z53 females choosing HG males over Z53 males is ≈0.007 ($= e^{-4.8}$).

Cross 5 shows that males from the intermediate Z′ line (Z29) are almost equal to Z/H hybrid males in their mating with both Z and C females. Cross 6 and 7 of Table 4 show F_1 hybrid females to be much less discriminant than pure Z females, consistent with the no choice experiment of Table 3. The near absence of discrimination in cross 7 is puzzling because these females are zygotically identical with those of cross 6. (If anything, females of cross 7 should be more Z-like because of the maternal influence from their Z mothers.)

To further analyze the genetic basis of male mating behavior, we carried out whole-chromosome substitutions by F_2 backcross (see Table 3). In this scheme, only certain substitution types could be obtained. Because eye color mutations were used to track chromosome, comparisons have to be between males of the same eye phenotype, which are juxtaposed in Table 3, set b. Between cross 7 and 8, it is shown again that the X chromosome has little effect on the mating success of males ($P > 0.1$). It is also clear from the comparisons between cross 10 and 11 ($P < 0.01$ for both 1-hr and 1-day observations) that the X and one copy of the second chromosome of Zimbabwe together bring about a certain degree of Zimbabwe maleness to the flies. This is also true for the combination of the X and third chromosome of Zimbabwe in cross 12 and 13 ($P < 0.01$ for both 1-hr and 1-day observations). Apparently, both autosomes carry genetic determinants for Zimbabwe maleness.

Postmating Isolation. There is no evidence for hybrid inviability or sterility in either F_1 or F_2 flies. We have dissected multiple males of F_1 and each of the four F_2 genotypes of Table 3, set b, to observe sperm development, as done in previous studies of hybrid sterility (20). Between all five Zimbabwe lines and selected strains from our *D. melanogaster* collection, hybrid male sterility is very low and sporadic. Moreover, all six possible homozygous whole chromosome substitutions between Z30 and HG (or FR) are fertile and viable (H.H. and C.-I.W., unpublished results).

Spread of the *P* Transposable Element. Because the transposable element *P* has been shown to invade all populations of *D. melanogaster* worldwide, including those thought to be geographically isolated from others in the recent past, as reported in ref. 21, it would be of interest to know if the Zimbabwe population contains *P* elements. Indeed, all five Zimbabwe lines were found to have 15–30 copies of *P* elements by genomic Southern hybridization. Horizontal transfer of *P* is not highly prevalent as the commensal sibling species, *D. simulans*, is not known to harbor this element at all (22). The result suggests that the influx of genes from *D. melanogaster* into the Zimbabwe population by mating in the last several decades is not absolute zero.

DISCUSSION

We show in this report that strong sexual isolation exists within the species of *D. melanogaster*. The results support the suggestion of strong population structure in African *D. melanogaster* (9). The intensity of this isolation is not only strong (nearly complete in one direction) but also surprisingly robust.

Table 4. Multiple choice experiments on hybrid males and females

$♀_A$	$♀_B$	$♂_A$	$♂_B$	(n_{AA},n_{AB}/n_{BA},n_{BB})	DI*
Male behavior (HG vs. H/Z vs. Z/H vs. Z)					
1. Z53 (60)	HG (53)	H/Z (56)	HG (54)	(22, 2, 18, 36)	3.09 ± 0.79
2. Z30 (74)	HG (62)	H/Z (64)	HG (59)	(34, 2, 24, 38)	3.29 ± 0.77
3. Z53 (58)	HG (60)	Z/H (48)	H/Z (54)	(22, 22, 27, 30)	0.11 ± 0.40
4. Z53 (64)	HG (50)	Z53 (52)	Z/H (57)	(34, 12, 17, 29)	1.58 ± 0.45
Male behavior (Z29 vs. Z/H)					
5. Z30 (49)	FR (44)	Z29 (34)	Z/H (42)	(12, 14, 16, 21)	0.12 ± 0.51
Female behavior					
6. H/Z (52)	HG (49)	Z53 (52)	HG (60)	(29, 10, 14, 34)	1.95 ± 0.49
7. Z/H (65)	HG (44)	Z53 (60)	HG (57)	(31, 30, 18, 26)	0.40 ± 0.40

See legend to Table 2. $♀_A$ and $♀_B$ are the numbers of females and $♂_A$ and $♂_B$ are the numbers of males of the designated type.
*Mean ± SE.

Substituting the chromosomes is the only way to change the behaviors.

The Zimbabwe populations may be at the incipient stage of speciation for the following reasons. (*i*) There is polymorphism in the genetic determinants of male and female sexual behaviors in Zimbabwe (as well as in nearby regions). (*ii*) There is no detectable hybrid sterility in F_1 or F_2 even though sterility is often a very sensitive measure of species divergence in *Drosophila* (23–25). (*iii*) The entire X chromosome has diverged very little with respect to mating behavior. On this last point, we wish to caution against the generalization, based on a few selected studies like this one, that sex chromosomes play a disproportionately smaller role in sexual isolation. Even among *Drosophila* studies, sex chromosomes have been shown to be much more important (5, 6), equally important (3, 4), or much less important (7) than autosomes. (For contrasting views on sex chromosome and postmating isolation, see refs. 24, 26, and 27.)

It has been reported before that flies from a Congo population exhibited some sexual isolation from those of a French population (ref. 28; see their double-choice experiments). Although these authors concluded "strong sexual selection but no reproductive isolation" on the basis of overall weak performance of the French males, the tantalizing question is whether the behavior they observed is related to the Zimbabwe phenomenon. Determining geographical distribution of this phenomenon will be crucial for elucidating its origin.

The absence of an obvious fitness reduction in F_1 and F_2 flies suggests that sexual isolation in this case might not have evolved by reinforcement (29, 30). There are several models attempting to explain speciation by sexual selection (31–33). In these models, it is generally assumed that female choice is the dominant factor (34). Our observations are compatible with this assumption. (*i*) The failure of Z × C mating cannot be accounted for by Z males interfering with C males because of the same result in the no choice experiments. (*ii*) C males do court Z females as frequently as they court C females in male choice experiments (unpublished results). (*iii*) C males' success depends on what other choices Z females may have. For example, hybrid males (H/Z or Z/H) account for >90% of the matings by Z females in cross 1 of Table 4 when the other choice is HG males, whereas they account for only 25% of the matings by the same Z females in cross 4 when the females can choose to mate with Z males. In the absence of direct male–male interference, females' decision must have played an important role.

A second element in the models of speciation by sexual selection is that the male and female behavior genes would be in gametic phase disequilibrium, whether they are linked or not (32, 33). In other words, a strain is expected to be either C or Z for both sexes. Thus, the presence in moderate frequency of intermediate Z′ strains (Z29 and LA69, Table 2) in nature can potentially be problematic. Females of Z29 are very much C-like in their preference but males are more Z-like in their mating success. The possible discrepancy cannot be resolved until its genetic basis is understood. One possibility for the intermediate behavior in isofemale lines is that it represents a mixture of two pure types. In that case, it should be possible to extract isochromosomal lines that exhibit either Z or C behavior. A stable polymorphism coupled with strong linkage disequilibrium would be an indication that two reproductively isolated groups coexist in the same population. Theoretically, speciation by sexual selection could occur sympatrically if the population size is sufficiently large (33). The reported sexual isolation in *D. melanogaster* in Africa offers a rare opportunity for testing such hypotheses about the genetics of speciation.

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