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INCIPIENT SPECIATION BY SEXUAL ISOLATION IN *DROSOPHILA MELANOGASTER*: VARIATION IN MATING PREFERENCE AND CORRELATION BETWEEN SEXES

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Abstract.—Genetic divergence for characters pertaining to reproductive isolation is of considerable interest in evolutionary biology. Since most studies concentrate on sibling species (for recent reviews, see Wu et al. 1996), we would like to know how much genetic variation exists between populations that are at an incipient stage of speciation. To answer this question, we have begun measuring variations in mating preference among natural isolates of *Drosophila melanogaster*, represented by the cosmopolitan and Zimbabwe sexual races. We quantify the variation in mating preference and success in both sexes by using a multiple-choice design and an index that is suited to cases of strong asymmetry in mate choice. Different designs and indices for measuring sexual isolation are also discussed. These sexual traits are entirely genetically determined. Surveying four populations in southern Africa and additional cosmopolitan lines, we observe extensive genetic variation in sexual characters as well as strong correlation between sexes. The populations are highly differentiated and represent various stages of evolution between the African and the cosmopolitan type of sexual behaviors. The genetic variation and correlation for these sexual characters coupled with their geographical pattern have interesting implications for models of speciation by sexual selection.

Key words.—Genetic variation, population differentiation, sexual selection.

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There has been progress in the last decade in understanding the evolution of reproductive isolation. (e.g., Hollocher and Wu 1996; True et al. 1996; Palopoli et al. 1996; for reviews, see Wu and Palopoli 1994 and Wu et al. 1996). A main conclusion is that the genetic divergence underlying postmating isolation between sibling species is very extensive. No fewer than 120 loci contribute to the hybrid male sterility between *Drosophila simulans* and *D. mauritiana* (Davis and Wu 1996). These two species are probably beyond the incipient stage of speciation (Tsacas and David 1974). Nevertheless, because they still share many ancient polymorphisms, the genetic distance between them at the DNA sequence level is still quite small (Hey and Kliman 1993).

What is, then, the level of genetic divergence between local populations that are at an incipient stage of speciation? Comparing *D. melanogaster* collected from Zimbabwe with the cosmopolitan lines is an ideal system for this question. Begun and Aquadro (1993) first reported that a Zimbabwe population has diverged from the North American collection at the molecular level. Further study showed that females of many lines collected in Zimbabwe have a nearly exclusive preference for males from the same locale over males from other regions of Africa or other continents (Wu et al. 1995). Females from those other regions, on the other hand, do not show selectivity in choosing mates. Clearly, both female and male sexual characters have diverged among these populations (we use “sexual characters” as a general term for both female preference and male mating success). We designate the two behavioral types as the Z-type (for Zimbabwe) and M-type (for *melanogaster* of the cosmopolitan type). Being strictly governed by their chromosomal genotypes, these mate choices are highly stereotypic and robust under many different environmental and physiological conditions (Hollocher, Ting, M. Wu, and C.-I. Wu, submitted ms.)

In addition to the absence of obvious postmating isolation, *within-population* variation in the Z-type versus M-type behaviors may be a signature of incipient speciation (Wu et al. 1995). The need to survey the level of within-population variation motivated this study. Since the level of postmating isolation is too low to detect, we may also learn from this system how much genetic divergence underlying sexual isolation can evolve in the absence of reinforcement (Dobzhansky 1970; Kelly and Noor 1996). In this report, we survey populations in southern Africa and analyze the extent of polymorphisms in sexual characters of both sexes. By comparing the level of such polymorphisms across populations, we are able to describe the trend of evolution in sexual preferences in this species.

MATERIALS AND METHODS

Isofemale Lines and General Stock Keeping

The Zimbabwe isofemale lines were collected from Sengwa Wildlife Reserve (ZS) in 1990 (Begun and Aquadro 1993) and near Harare (ZH). Both sets of samples were from C. F. Aquadro. The Luangua (LA; Rob Dorit, pers. comm.) lines are from northern Zambia and the Okavanga (OK) lines are from Botswana. Each of the five cosmopolitan lines, FR (France), HG (California), Tai (Ivory coast, J. David), TWN (Taiwan), Yep-11 (Australia, I. Boussy) is from a different continent. The locations of the African collection are shown in Figure 1. We designate the cosmopolitan lines as the M-type, instead of the C-designation used previously (Wu et al. 1995), because the C-Z designation has become unwieldy in the genetic analysis. Flies are kept in a constant temperature room (23°C) with humidity control and a 12h light/dark cycle.

Mating Experiments

We used a multiple-choice design in which females and males of both lines could choose their mates. Virgin females

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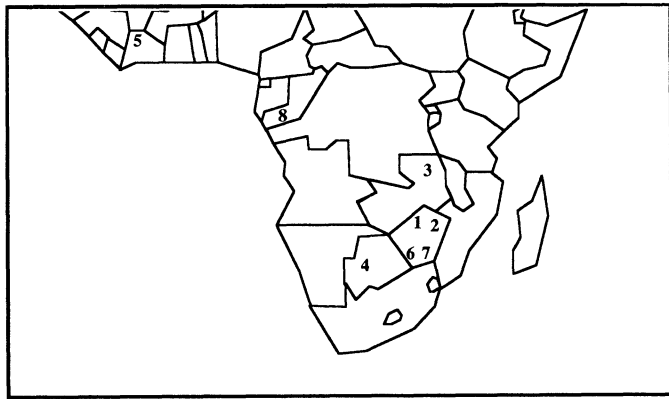


FIG. 1. The locations from which isofemale lines were collected. (1) ZS; (2) ZH; (3) LA; (4) OK; and (5) Tai, as presented in Table 1. (6, 7) are the locations represented in Dubill (1996); and (8) is the location in Cohet and David (1980).

and males of the two tested strains were simultaneously released into a population cage. Usually, 55–65 flies of each sex of each genotype were used (thus, 220–260 flies were in a cage). Flies of different strains were fed different food coloring prior to experimentation and each copulating pair was aspirated out of the cage for type identification. This design seems to be closer to the natural setting than the no-choice or single-sex choice experiments. Large sample size is another advantage; an experimenter can observe more than 100 copulations in a one-hour period. We have also found that measuring relative mate choice in multiple-choice experiments yield more consistent results than the single-choice or no-choice design. Experiments were always started right before the beginning of the light cycle. For details, see Wu et al. (1995).

Analysis of the Mating Pattern by the Discrimination Index (DI)

Given the multiple choice results, there are several isolation indices (e.g., Levine 1949; Malokolowkin-Cohen et al. 1965) to analyze them. Here we present a (relative) discrimination index that is additive and is independent of the input numbers in the experiments. Let f_A and m_A be the number of females and males from strain A, and let f_B and m_B be the number of females and males from strain B that are released into a cage for mating. Let the observed numbers of mating be: n_{AA} (A-females \times A-males), n_{AB} (A \times B), n_{BA} (B \times A), and n_{BB} (B \times B). The success of B males in mating with A females, relative to that of A males, is $W = (n_{AB}/f_A m_B)/(n_{AA}/f_A m_A)$. The relative success of these males in mating with B females, likewise, is $V = (n_{BB}/f_B m_B)/(n_{BA}/f_B m_A)$. It is easy to see that $W/V (= [n_{BA} n_{AB}]/[n_{AA} n_{BB}])$ indicates A-females' acceptance of B-males over A-males, relative to B-females' acceptance of the same two types of males. W/V is an index of females' relative preference for the two types of males. We shall define the Discrimination Index (DI) by the logarithmic transformation of W/V because it is easier to obtain the variance estimate on the transformed formula. Thus,

$$DI = -\ln(W/V) = -\ln([n_{BA} n_{AB}]/[n_{AA} n_{BB}]). \quad (1)$$

$DI = 0$ ($W/V = 1$) means there is no difference in mating preference between these two types of females and a positive value means that homogamic matings are more frequent than expected. When n_{AB} or n_{BA} is zero, DI becomes ∞ . In those cases, one is substituted for zero to yield a conservative estimate of the strength of mate choice. Note that DI can be rederived by assuming male preference as well. Results from multiple-choice experiments are compatible with either male or female preference, but female preference is strongly supported by additional observations (Wu et al. 1995) including equal male courtship of the two types of females (unpubl. behavior obs.). By assuming that the mating preference for either type of mates is binomial, we obtain the variance estimate of DI by the Delta method as $\text{Var}(DI) = 1/n_{AA} + 1/n_{BA} + 1/n_{AB} + 1/n_{BB}$. The variance formula was first given by Fisher and rederived by Maruyama and Crow (1975) in their viability study. Because only binomial variation is taken into account, the actual variance may be larger.

There are three reasons to use DI to analyze multiple-choice experiments. First, DI measures the *relative* strength of mate choice between two test strains and is independent of the input values, m_A , m_B , f_A , and f_B . Although it is the standard practice to have $m_A = m_B$, in reality, it is not possible to know if the two types of males are equally vigorous. Thus if A-females favor A-males over B-males, given $m_A = m_B$, it is not clear if B-males in that particular experiment were simply less vigorous, due to experimental variables, or females truly prefer A-males. Such variables include culture conditions, crowding, bacterial infection, handling, anesthetizing, and so on. Another important reason to factor out m_A , m_B , f_A , and f_B is that these numbers decrease as mating takes place and the more successful type decreases more rapidly. The apparent preference of M-females for M-males (Table 1) in part reflects the gradual increase in the proportion of unmated M-males. DI is a measure of the relative mating preference between the two types of females and should be independent of the relative health or the number of males remained unmated during the course of the experiment. For this reason, we do not use a female-choice design, which often yields a few Z-female \times M-male matings when the ratio of Z:M males becomes low, thus biasing the estimate of female preference. Second, DI is more suited to measuring asymmetric sexual isolation, whereas other indices are more concerned with the average of isolation in both directions. For example, in the Malokolowkin-Cohen index (1965, $[n_{AA} + n_{BB} - n_{BA} - n_{AB}]/N$, where N is the total number), there is no difference between $(30 + 20 - 0 - 40)/90$ and $(30 + 20 - 20 - 20)/90$, but the former shows very strong asymmetric isolation. (In contrast, the DI values for the two cases are, respectively, ∞ and 0.405.) Third, DI is nearly additive when used to measure the degree of divergence in male behavior. This advantage is apparent in the genetic analysis (Hollocher, Ting, M. Wu, and C.-I. Wu, submitted ms.).

Measuring Behavioral Differentiation between the Unknown and the Standard Lines

Given any unknown isofemale line, U, we measure its sexual isolation from the standard M-line (FR and/or HG) and from the standard Z-line (Z30 and/or Z53). Isolation from

TABLE 1. Discrimination index (DI) between the tested line (U) and pure Z- or M-lines, $DI(U, M)$ and $DI(Z, U)$, as illustrated in Figure 2. The observed numbers of copulations for measuring $DI(U, M)$ are given as $(n_{AA}, n_{AB}, n_{BA}, n_{BB})$ where A designates U, and B designates M. n_{AB} stands for the number of matings between A-females and B-males. The same applies to $DI(Z, U)$ measurements where A designates Z, and B designates U. The collection sites are given in Figure 1 and the materials and methods section. When $n_{AB} = 0$, one is substituted for zero in the calculation of DI.

Lines	$DI(U, M)$ ($n_{AA}, n_{AB}, n_{BA}, n_{BB}$)	$DI(Z, U)$ ($n_{AA}, n_{AB}, n_{BA}, n_{BB}$)
Southern African lines		
ZS 2	5.14 (34, 0, 12, 60)	0.87 (49, 33, 15, 24)
ZS 6	3.49 (26, 5, 7, 44)	1.86 (36, 10, 14, 25)
ZS 8	3.06 (54, 18, 10, 71)	1.72 (42, 5, 39, 26)
ZS 11	4.38 (33, 0, 28, 68)	-0.40 (39, 51, 32, 28)
ZS 28	2.01 (86, 22, 35, 67)	1.19 (38, 18, 36, 56)
ZS 29	1.05 (11, 7, 11, 20)	1.58 (47, 12, 46, 57)
ZS 30	4.81 (64, 2, 25, 96)	-1.02 (26, 26, 25, 9)
ZS 32	5.76 (68, 1, 16, 75)	0.79 (49, 32, 27, 39)
ZS 40	2.71 (116, 31, 23, 92)	2.11 (88, 26, 34, 83)
ZS 49	5.91 (37, 1, 4, 40)	0.22 (30, 17, 24, 17)
ZS 53	4.22 (48, 0, 34, 48)	-1.02 (26, 26, 25, 9)
ZS 56	4.15 (34, 0, 42, 78)	-0.45 (24, 23, 18, 11)
ZH 12	4.55 (78, 3, 16, 58)	0.50 (51, 37, 26, 31)
ZH 16	3.04 (28, 3, 26, 58)	0.96 (59, 35, 20, 31)
ZH 27	0.07 (29, 31, 35, 40)	2.07 (41, 7, 39, 53)
ZH 32	4.25 (33, 0, 31, 66)	0.63 (30, 16, 27, 27)
ZH 33	1.47 (34, 13, 18, 30)	0.16 (38, 47, 11, 16)
ZH 34	4.10 (54, 2, 25, 56)	0.61 (38, 48, 24, 56)
ZH 42	2.45 (63, 8, 40, 59)	1.29 (67, 32, 22, 38)
OK 17	0.31 (43, 21, 9, 6)	4.73 (46, 0, 15, 37)
OK 59	2.64 (96, 15, 26, 57)	1.64 (27, 10, 20, 38)
OK 87	4.47 (39, 0, 16, 36)	-0.25 (19, 19, 9, 7)
LA 2	1.99 (47, 29, 14, 63)	3.49 (47, 2, 28, 39)
LA 20	0.26 (13, 15, 12, 18)	3.02 (19, 0, 12, 13)
LA 47	0.18 (18, 15, 17, 17)	3.01 (16, 0, 15, 19)
LA 66	0.44 (17, 11, 12, 12)	3.49 (19, 3, 5, 26)
LA 69	2.92 (48, 4, 44, 68)	0.45 (27, 9, 23, 12)
LA 79	2.61 (24, 6, 5, 17)	0.93 (14, 11, 5, 10)
Cosmopolitan lines		
FR	1.12 (27, 25, 12, 34)	4.71 (65, 0, 38, 65)
HG	1.12 (34, 12, 25, 27)	4.48 (47, 2, 21, 79)
Tai	0.91 (36, 17, 23, 27)	3.71 (31, 2, 17, 45)
TWN	0.12 (27, 27, 23, 26)	3.49 (35, 3, 15, 42)
Yep 11	1.19 (50, 41, 24, 65)	2.80 (33, 4, 15, 30)

the M-line and Z-line is designated $DI(U, M)$ and $DI(Z, U)$, respectively, as illustrated in Figure 2. Although one may think that the two measures are redundant, or at least highly negatively correlated, they in fact measure distinct sex-dependent properties. On the scale of Figure 2, a large $DI(U, M)$ would suggest that the U-line is Z-like. That is because U-females would not mate with M-males and hence are Z-female-like. On the other hand, a small $DI(Z, U)$ also indicates the unknown line to be Z-like but that is due to the success of U-males in mating with Z-females, (i.e., U-males are Z-like). Thus, $DI(U, M)$ reflects primarily how readily females of the U-line would mate with M-males and $DI(Z, U)$ measures primarily the degree to which males of the U-line would be successful in mating with Z-females. Three additional observations support this interpretation. (1) The deviation from random mating in this system is mainly due to female choice. If male choice is operating, it would be much weaker in comparison with female choice (Wu et al. 1995).

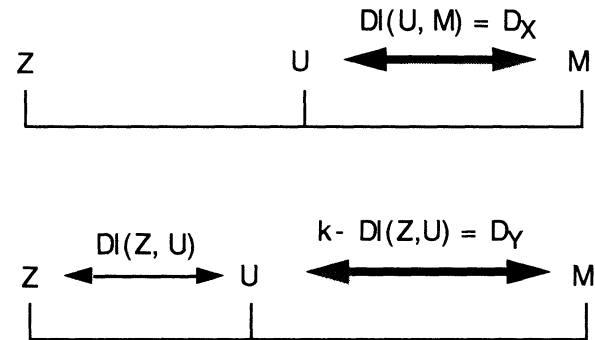


FIG. 2. A schematic representation of the measurement of sexual isolation between any isofemale line with unknown sexual behaviors (U) and the pure Z- or pure M-lines. D_X measures the divergence between the U-line and a given M-line, primarily as a function of how Z-like females of the U-line are. Similarly, D_Y is the divergence between the U-line and a given M-line, primarily as a function of how Z-like males are. The actual measurements of $DI(U, M)$ and $DI(Z, U)$ are given in Table 1 for 28 different isofemale lines; k is a constant. We use $k = 5$ to represent $DI(Z, M)$.

(2) M-females do not discriminate between males of the Z- and M-type (or the intermediate Z' -type). When M-females alone were given any two types of males, they mated randomly (Ting and Wu, unpubl. data). This property also makes M-females a good choice as an internal control in the multiple-choice design. The apparent bias in M-females' mating reflects the ever-changing relative availability of the two types of males. (3) Pure Z-females' rejection of M-males is nearly complete. Even between partial Z-types and pure M-males, the former still account for all the matings with Z-females (Hollocher, Ting, M. Wu, and C.-I. Wu, submitted ms.).

Since a large $DI(U, M)$ and a small $DI(Z, U)$ both suggest Z-like sexual characters (in males and females, respectively), it is more intuitive to compare $DI(U, M)$ with $k - DI(Z, U)$ where k is a constant. We may let k be $DI(Z, M)$, as shown in Figure 2, although any constant would suffice in the ANOVA and regression analysis. (We use $k = 5$, which is close to the average value of $DI[Z, M]$ s.) We designate D_X (divergence between U and M due to female preference) for $DI(U, M)$ and D_Y (divergence between U and M due to male mating success) for $k - DI(Z, U)$ in Figures 2 and 3. Note that, statistically, D_X and D_Y do not have to be correlated. For example, an unknown isofemale line could have females with M-like preference but males with Z-like traits. D_X ($= DI[U, M]$) would then be 0 and D_Y ($= k - DI[Z, U]$) would have the maximum value of k . Therefore, a positive correlation must reflect an underlying genetic correlation between male and female characters.

In Table 1, we use FR or HG as the standard M-line and Z30 or Z53 as the standard Z-line. We replicate some of these measurements by using both M-(or Z-) lines. We have applied several procedures to combine the two sets of data, including averaging the DI-values and testing for heterogeneity. Since adding all the n_{ij} values before computing the final DI is more desirable for cases where the mate choice is extreme (notably, $n_{AB} = 0$) and the different procedures yield similar results, we only present the sums of the observed n_{ij} -values in Table 1, when a strain was tested against both standard lines.

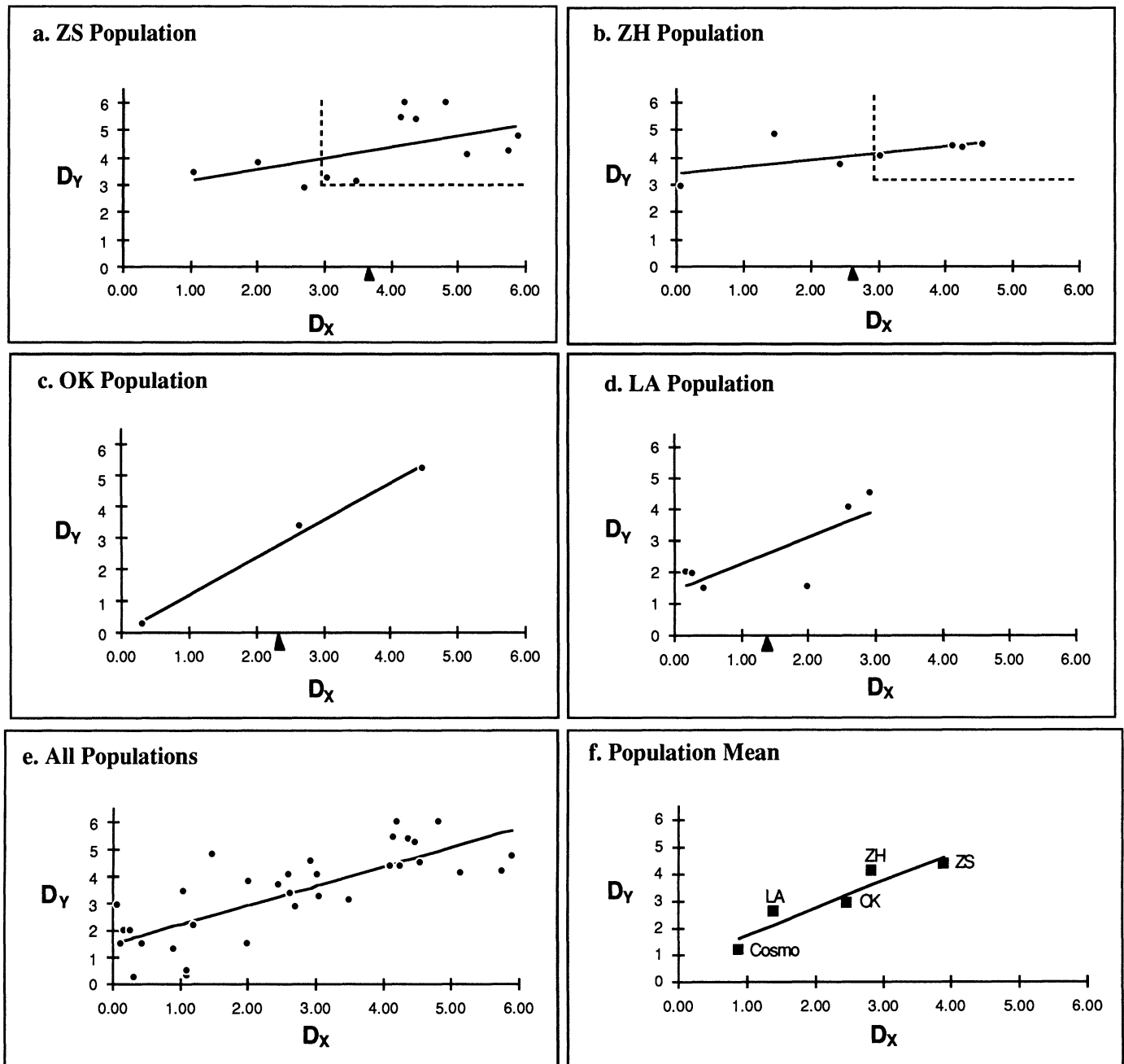


FIG. 3. Regression analysis of D_Y on D_X . Each dot denotes an isofemale line and each square denotes the population mean. (a–d) Analysis of each African population. The upper-right corner outlined by the broken lines is the area of strong Z-type. Solid triangle indicates the mean Z-femaleness of that population. (e) Analysis of the entire species, consisting of the four populations of (a–d) and the five cosmopolitan lines. (f) Regression of the population mean D_Y on the population mean D_X .

RESULTS

Twenty-eight isofemale lines from four populations in southern Africa (Fig. 1) as well as five lines from a cosmopolitan collection were analyzed. Each isofemale line is characterized for its divergence from both the Z- and M-type. The divergences depend primarily on how Z-like their females (D_X) and males (D_Y) are (Fig. 2). In general, $D_X > 3$ or $D_Y > 3$ indicates strong Z-type behaviors. In the loga-

rithmic scale, such a threshold represents a 20-fold difference in the relative mating preference.

The results of our survey are given in Tables 1, 2 and plotted in Figure 3. Our observations indicate that the species of *D. melanogaster* as a whole harbors extensive genetic variation in sexual behaviors. This contrasts with an earlier conclusion (Henderson and Lambert 1982) because African samples were underrepresented in their study. In our survey, the Zimbabwe collections (ZS and ZH) show the highest fre-

TABLE 2. Summary statistics of D_X s and D_Y s for the five populations. The regression analysis is for D_Y on D_X as shown in Figure 3; n is the number of isofemale lines from each population.

Population	n	D_X		D_Y		Regression		Correlation	
		Mean	Variance	Mean	Variance	Slope	Intercept	r	r^2
ZS	12	3.89	2.20	4.38	1.28	0.411	2.780	0.540	0.292
ZH	7	2.85	2.70	4.11	0.40	0.235	3.442	0.612	0.375
OK	3	2.48	4.35	2.96	6.33	1.203	-0.016	1.000	0.995
LA	6	1.40	1.57	2.61	1.82	0.844	1.418	0.783	0.613
Cosmopolitan	5	0.89	0.20	1.16	0.59	-0.379	1.500	0.217	0.047
Total	33	2.63	3.17	3.38	2.66	0.710	1.513	0.775	0.601

quency of Z-type behaviors. The frequency then decreases in the LA collection to the north and the OK collection to the west. Both of these populations harbor pure M-type or near-M-type males ($D_Y < 2$; males strongly discriminated by standard Z-females), which are absent from the two Zimbabwe collections. Indeed, the means in D_Y are high and their variances are low in the Zimbabwe populations because their females would overwhelmingly prefer to mate with Z-males. The OK collection is intriguing in that the entire spectra of sexual behaviors are represented by merely three lines. This population may be most variable with respect to sexual behaviors. The four cosmopolitan lines and the one western African line are all unambiguously M-type. Dubill (1996) has also surveyed a small number of lines from many more locales in Zimbabwe (see Fig. 1). His collections from southern and western Zimbabwe resemble the LA population reported here. If we include the single line from Congo (Cohet and David 1980), which may be Z-like, the Z-versus M-type behavioral polymorphism appears widespread in southern Africa.

To see whether the cosmopolitan collection is differentiated from the southern African populations, we carried out the simple t -test between the five cosmopolitan lines and the 28 African lines on both D_X and D_Y . For D_X , $P = 6.5 \times 10^{-6}$ and for D_Y , $P = 6.6 \times 10^{-5}$. Clearly, *D. melanogaster* from this part of Africa are strongly differentiated from the cosmopolitan flies in their sexual preferences. We also carried out one-way analysis of variance (ANOVA) to see if the four African populations have differentiated. For both D_X and D_Y , $P < 0.05$. Although ANOVA assumes constant variance, it has been shown that a fixed-model ANOVA is robust when the variance is not constant (Anderson and McLean 1974; Milliken and Johnson 1992). (Because the D_Y variance in the ZH population is much smaller than those of other African populations, we also removed this population from the ANOVA test and the results were essentially the same [$P < 0.05$].) Therefore, there appears to be some degree of population differentiation in parts of Africa with respect to mating behaviors. In contrast, the rest of the world seems relatively homogeneous in this regard (Henderson and Lambert 1982).

Another interesting aspect of the data is the correlation between D_X and D_Y within each population. Correlation and regression analyses are summarized in Table 2 (see also Fig. 3). There is a positive correlation in each of the African populations (the coefficients are smaller in the Zimbabwe populations because of their low level of variation). For the species as a whole, about 60% of the variation in D_Y is

explainable by the variation in D_X ($r^2 = 0.60$). D_X and D_Y measure the genetic differentiation between isofemale lines and among populations. Their correlation reflects a genetic association between genes that give females Z-like preference and genes that render males Z-like in their mating success.

Not only is there differentiation among local populations with respect to their mating preference, but the manner of differentiation might also have been predicted, as shown in Figure 3f. Primarily, the mean D_X s and D_Y s of these populations appear to have been coevolving. In Figure 3f, we may be witnessing various stages of evolution between Z-type and M-type behaviors as represented by the different populations. Since the molecular and biogeographical data (David and Capy 1988; Lachaise et al. 1988; Singh and Long 1992; Begun and Aquadro 1993, 1995) suggest the cosmopolitan populations to be recently derived from Africa, the Z-type behaviors may be representative of the ancestral state from which the M-type behaviors have evolved and subsequently spread throughout the world. For this to happen, we propose that Z-like female preference has evolved toward M-like female preference slightly ahead of the relaxation of Z-maleness toward M-maleness. These initial stages are represented by the ZS and ZH populations (Fig. 3, Table 2). For example, the ZS population is still strongly Z-like although some of its females are moderately receptive to pure M-males. The ZH population, on the other hand, has evolved more toward M in female preference; some females are completely M-like in preference but their frequency is still too low for pure M-type males to exist in the population. The LA and OK populations and perhaps those of southern Zimbabwe (Dubill 1996) are a step further in this evolution with even weaker Z-female preference, which is accompanied by the noticeable loss of Z-maleness. Finally, the cosmopolitan populations are completely M-like in both sexes. Throughout the course, the sexual behaviors of the two sexes appear to be coevolving.

DISCUSSION

Several questions are brought to light by this survey: (1) What is the genetic basis underlying the polymorphisms in sexual characters in this species? (2) How are the polymorphisms maintained in southern Africa? (3) Why are these polymorphisms absent from other continents? (4) In what direction and by what mechanisms have these polymorphisms been evolving?

Question 1 is the main focus of Hollocher, Ting, M. Wu, and C.-I. Wu (submitted ms.). In that study, it is shown that

the nonrandom mating between Z- and M-types as measured in the multiple-choice experiments is not much affected by environmental factors. Chromosome substitution is the only way to alter the mating pattern and each of the two major autosomes harbors strong genetic determinants, which will make further dissection at the genic level possible.

Question 2 implies that there is a cost to being the Z-type because, if everything else being equal, the mating advantage of Z-type over M-type males should have driven the Z-type genes to fixation. Among others, there are indications that Z-type females may have reduced fecundity (D. Rand, pers. comm.). Most importantly, it is necessary to show that the fitness reduction is directly associated with genes governing sexual characters. In systems that are not as amenable to genetic analysis, it is common to try to demonstrate that the phenotype itself (say, female choice) incurs a cost. However, if the cost is derived from pleiotropic effects unrelated to the phenotype itself, the task would not be feasible. In the Z-M system, it is possible to find the cost associated with both the Z-phenotype and Z-genotype. Genetic analysis coupled with laboratory population experiments (cf. Prout 1971) that attempt to unravel selection components may be promising.

Question 3 may imply that the cost with the Z-type becomes even higher outside of Africa. Cohet and David (1980) have earlier suggested that *D. melanogaster* in Africa should be more discriminatory because there are many more closely related species on that continent. Therefore, the higher receptivity threshold observed in Z-females may be expected. The hypothesis assumes that *D. melanogaster* cohabit with other species extensively in the wild of southern Africa. The hypothesis also implies that the diversity of related species, not the density of heterospecific flies, drives the evolution of female receptivity since *D. melanogaster* are often found together with *D. simulans* at high density in worldwide collections. One prediction of this hypothesis is that Z-type females would be more discriminatory toward heterospecific males than M-type females. While these assumptions and predictions will need to be tested, answers to question 4 may also provide a solution to question 3.

With respect to question 4, the Z-type behaviors may represent the ancestral state based on the molecular and biogeographical data, as discussed in the Results. This direction is somewhat paradoxical in that the newly evolved M-male-ness actually puts males at a mating disadvantage when Z-type females are present. The evolution of the less successful M-type males seems to be the reversal of the runaway process (Fisher 1930; Lande 1981; Kirkpatrick 1982). The direction of evolution from the Z- to M-type does agree with Kaneshiro's prediction (1983) that the presumed ancient female type (Z) discriminates against the derived male type (M); however the underlying mechanisms may be quite different. In Kaneshiro's model, changes in the pattern of mating occur through a relaxation of sexual selection brought on by a founder event. During a founder event, selection against females that are highly discriminatory in mate choice occurs resulting in a shift in the frequency of different mating types in the population (Kaneshiro 1989). Although this hypothesis is appealing because it explains why derived females tend to be less choosy, its reliance on severe bottlenecks to trigger the shift in mating systems may not be applicable to *D. mel-*

anogaster. In addition, studies on other species of this subgroup show the evolution of mating discrimination to have a pattern opposite that of Kaneshiro's prediction (Watanabe and Kawanishi 1979).

A more recent model (Iwasa and Pomiankowski 1995) addresses this paradox in a different way. Briefly, the self-reinforcement mechanism of the runaway process would drive the female preference and male trait to the limit and then, at a certain point, weak selection against female mate choice (which relates to question 2 above) pushes the population onto a "sliding-back" path that takes a longer time to return than to get there the first time. Hence, one is more likely to encounter populations on the return path, which could help account for why derived populations are more often associated with a relaxation in female preference. The current populations could represent various stages of the sliding-back phase of that evolution, regressing toward the M-state. According to this model, one does not have to postulate that the cost of Z-type varies in different geographical areas. Instead, different populations are transient at different stages of evolution. The model in its current form is symmetric in that M-females should prefer M males as strongly as Z-females prefer Z-males, but females' preferences in our observations are highly asymmetric. It would be interesting to see whether M-females who strongly prefer M-males over Z-males could be selected from the mixture of worldwide collections.

The excitement about sexual isolation in the species of *D. melanogaster* stems from the opportunities of detailed genetic dissection, as has been done for postmating isolation (Wu and Palopoli 1994; Hollocher and Wu 1996; True et al. 1996). In the near future, it should be possible to study the geographical distribution of genes at the loci of sexual isolation, instead of the phenotypes as is done here. The differentiation of *D. melanogaster* populations with respect to sexual behavioral genes would be particularly informative about speciation at the incipient stage (Palopoli et al. 1996). It may also be possible to measure the fitness effects associated with different alleles of sexual behaviors. For example, long-term evolution of the sexual behavioral polymorphisms can be simulated in laboratory populations. The system has the potential to combine the genetic studies of speciation with sexual selection theories into a coherent field of investigation.

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