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Genetic basis of sexual isolation in *Drosophila melanogaster*

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Abstract

Sexual isolation between Zimbabwe (abbreviated as Z) and cosmopolitan (abbreviated as M) races exists in *Drosophila melanogaster*. Typically, when given a choice, the females from the Zimbabwe race mate only with males from the same race, whereas females from the cosmopolitan race mate readily with males from both races non-discriminatorily. Genetic tools available in this experimental organism permit the detail genetic analyses of the sexual isolation behavior. On the other hand, the search for the actual signaling systems involved in the mate recognition is still limited in this system. In this paper, we review the studies, which attempt to dissect the genetic basis of the sexual isolation system, and document the complex features of the genetic architecture and the behavioral traits that evolved at an incipient stage of speciation. The evolution and the maintenance of this behavioral polymorphism are also discussed.

Abbreviations: Z – Zimbabwe; M – cosmopolitan.

Introduction

Genetic basis of the traits involved in speciation is essential for modeling the process of speciation (Nei, Maruyama & Wu, 1983; Gavrilets, 1999; Dieckmann & Doebeli, 1999; Kondrashov & Kondrashov, 1999). Intensive efforts to find the 'speciation genes' (reviewed in Coyne, 1992; Coyne & Orr, 1998; Wu & Palopoli, 1994; Wu, 2001) are often complicated by the problem of identifying which genetic changes have actually 'caused' the speciation from those that have accumulated during the species divergence after the initial speciation event. The exact evolutionary scenario maybe impossible to know, however, genetic changes in a system at an incipient stage of speciation should represent at least some of the early changes occurred during the process. The sexual isolation system between the Zimbabwe and the cosmopolitan races of *Drosophila melanogaster* provides an opportunity for the detail analyses of such process. Here, we review the studies, which attempt to genetically dissect this sexual isolation system, and to document the complex features of its genetic basis and the behavioral traits involved.

Sexual isolation between the Zimbabwe and the worldwide races

The first documentation of the existence of the strong sexual isolation between the *D. melanogaster* population in Zimbabwe and populations of other continents is in Wu et al. (1995). They reported that typically, in double-choice experiments, the females from the Zimbabwe (abbreviated as Z) population mate only with males from the same race, whereas females from the cosmopolitan (abbreviated as M) population show no preferences at all. The collection sites of the typical Z and M isofemale lines are shown in Figure 1 along with their relative intensities of the mating preferences (see below for detail). The existence of this

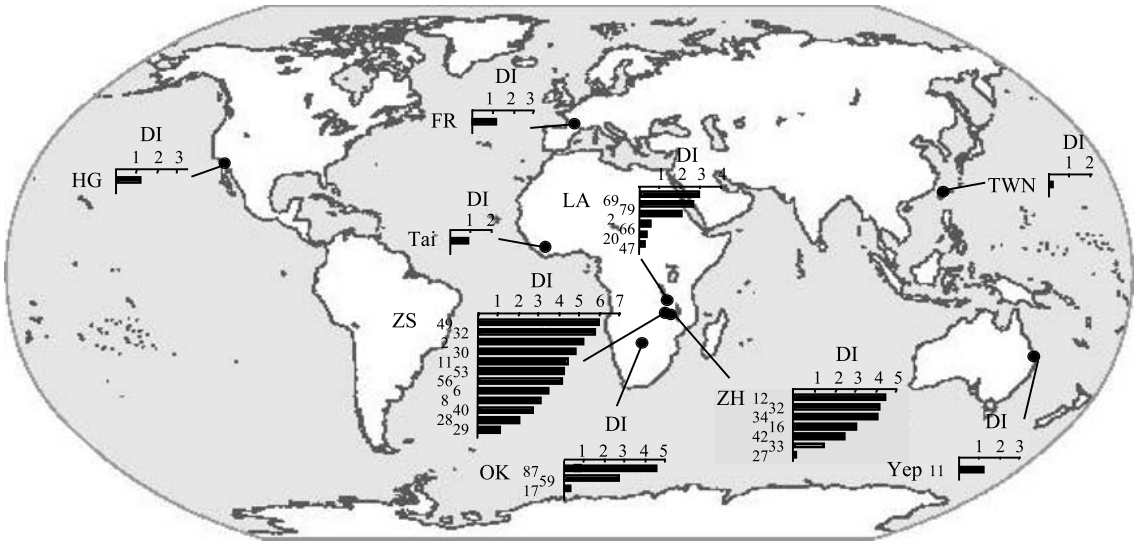

Figure 1. Worldwide distribution of the relative intensities of the Z/M behavioral traits. The discrimination indices (DIs) between the tested isofemale lines and a pure M-line are presented as histograms next to their sampled geographic locations. The index measures primarily how Z-like females the tested lines are (see Hollocher et al., 1997b for detailed formula). The names of the isofemale lines are shown next to the histogram. More than one isofemale lines from ZS, ZH, LA, and OK populations are indicated by different identification numbers. The data are from Hollocher et al. (1997b).

reproductive isolation was first inspired by an earlier report on nearly fixed nucleotide differences (i.e., high F_{ST}) between the Zimbabwe and USA samples of *D. melanogaster* in several loci on the X chromosome (Begun & Aquadro, 1993).

Why is this system unique? First of all, Wu et al. (1995) have argued that the system is most likely at an incipient stage of speciation for the following reasons. (i) There is polymorphism in the genetic determinants of male and female sexual behaviors in Zimbabwe and the nearby regions. (ii) There is no strong hybrid sterility in F_1 or F_2 even though sterility is often a sensitive measure of species divergence in *Drosophila* (Wu, 1992; Wu & Davis, 1993; Palopoli & Wu, 1994; Sawamura, Davis & Wu, 2000). (iii) Although the relative contribution of X chromosome to species divergence usually is larger than that of autosomes (Charlesworth, Coyne & Barton, 1987; Coyne & Orr, 1989; Laurie, 1997), the entire X chromosome has diverged very little with respect to mating behavior. Regarding the second point above, it should be noted that a recent finding by Alipaz, Wu and Karr (2001) showed some form of gametic incompatibility between Z and M flies. Nevertheless, other indices of postmating isolation including hybrid sterility seem to be not notable or very weak in this system. The last point also needs caution for interpretation as the 'large X effect' may not be significant in many premating isolation traits in general (Coyne & Orr, 1989; C.-I. Wu, personal communication).

Whether these behavioral traits would actually go to fixation in each population is not predictable at this point. However, it is this kind of intraspecific polymorphism in reproductive traits that has the potential to produce differentiating lineages. In this sense, we are observing the nascent stage of speciation.

Secondly, genetic tools available in *D. melanogaster* permit the establishment of well-defined and homogeneous recombinant lines to map the mating behavioral genes of Z and M. Since behavioral traits often have incomplete penetrance and variable expressivity, establishing homogeneous perpetual lines for various genotypes is desirable. Constructing these lines is feasible only with a whole set of genetic tools available in *D. melanogaster* such as balancer chromosomes. The studies that obtained a complete set of chromosome substitution lines or that constructed perpetual chromosomal segment substitution lines between two strains are all in this species (Breese & Mather, 1957, 1960; Hollocher et al., 1997b; Sureau & Ferveur, 1999). Despite many important studies done in systems of sexual isolation in other closely related species of *Drosophila* (Tan, 1946; Ehrman, 1961; Kawanishi & Watanabe, 1981; Zouros, 1981; Coyne, 1989; Noor, 1997), none of them has the above

advantage in increasing the resolution of genetic mapping to a certain level.

Finally, sexual selection is likely to be playing a role in the evolution and the maintenance of this behavioral polymorphism. Hollocher et al. (1997a) surveyed 28 isofemale lines from four populations in Africa for the intensity of the premating isolation. The distribution of the intensity of the Z/M behavioral traits indicated as DI (see below for definition) is shown in Figure 1 (data from Hollocher et al., 1997b). Their data showed extensive genetic variation in sexual characters and positive correlation between sexes.

Another case of partial prezygotic isolation in *D. melanogaster* has been reported between the two genetically differentiated populations in Brazaville, Congo (Capy et al., 2000). Comparisons of some genetically determined traits suggest that one of them consistently resembles European populations (Capy et al., 2000). Most likely, these two populations have been experiencing a secondary contact after one of them left Africa in the past, and so far, have been remained partially isolated from each other. Hence, the similar phenomena of sexual isolation between other Afrotropical and cosmopolitan populations may exist as well.

Genetic basis of the system

The large picture of the genetic architecture underlying Z/M behavioral traits has been obtained in Hollocher et al. (1997b). They constructed the whole set of chromosome substitution lines designated as ZMM, MZM, MMZ, ZZM, and ZMZ. Where, for example, ZMM refers to the genotype that is homozygous for the X chromosome of the Z-type and homozygous for the second and the third chromosome of the M-type. Typical Z-type and M-type isofemale lines were chosen as the two parental lines. By using appropriate balancer chromosomes that repress recombination, those whole-chromosome substitution stocks were constructed after seven generations of crosses (Figure 1 of Hollocher et al., 1997b).

Standard double-choice experimental design was used to test the genetic effect using those stocks (Wu et al., 1995; Hollocher et al., 1997a, b). Standard double-choice experiments to test for the female preference were done by releasing 55–65 flies of Z and M males and virgin females from two different chromosome substitution lines (test lines) into a population cage. Z and M females and males from two test lines were released when testing for the male mating character. Flies were fed with red- or green-colored food 1 day before the behavior test, and copulating pairs were aspirated out of the cage and scored by the color of their abdomens (details in Wu et al., 1995). To quantify the intensity of the effect, they calculated the discrimination index ($\mathrm{DI} = -\ln(n_{\mathrm{AB}}n_{\mathrm{BA}}/n_{\mathrm{AA}}n_{\mathrm{BB}})$, where n_{AA}, n_{AB}, n_{BA}, and n_{BB} are the observed numbers of mating between strains A and A (A females × A males), A and B (A females × B males), B and A (B females × A males), and B and B (B females × B males), respectively; Wu et al., 1995) from the scored numbers of the copulating pairs.

Many combinations of the substitution lines were tested in Hollocher et al. (1997b). From those mating cage data, the genes for the behavior were mapped to all three chromosomes with the same ranking for both sexes, which was III > II > X (III, II, and X designate the effects of the three chromosomes; Hollocher et al., 1997b). This whole-chromosome mapping already revealed extensive genetic divergence underlying this system. The fact that epistatic interactions and incomplete dominance were also detected in some lines (Hollocher et al., 1997b) suggested polygenic and complex genetic architecture. The following studies at a finer scale (Ting, Takahashi & Wu, 2001) strengthen this conclusion of the complexity.

In order to dissect the third chromosome that accounts for more than 50% of the total genetic effect on both male mating success and female preference (Hollocher et al., 1997b), Ting et al. (2001) created a series of recombinant lines at the subchromosomal level as described below that can be measured repeatedly.

In Ting et al. (2001), a multi-marker line (*rucuca*), which is M-type in mating characters, was used to construct recombinant lines for genetic analysis. This line carries eight visible markers on the third chromosome: roughoid (*ru*, 3-0, 61F5-62A3), hairy (*h*, 3-26.5, 66D15), thread (*th*, 3-43.2, 72B1), scarlet (*st*, 3-44.0, 73A3-4), curled (*cu*, 3-50.0, 86D1-4), stripe (*sr*, 3-62.0, 90E-F), ebony (*e*, 3-70.7, 93D2), and claret (*ca*, 3-100.7, 99B11-C1). The recombinants were generated between this multi-marker line and an MMZ line constructed by Hollocher et al. (1997b). After five generations of crosses using balancer chromosomes, the homogeneous recombinant stocks with their X and the second chromosome from an M source were established (Figure 1 of Ting, Takahashi & Wu, 2001). By comparing behavior of the recombinant lines

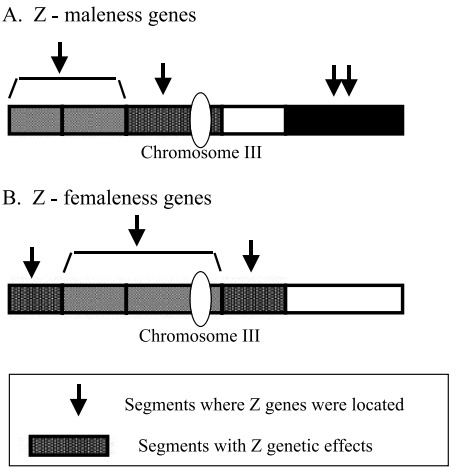

Figure 2. Chromosomal segments where the Z behavioral genes were mapped on the third chromosome of *D. melanogaster*. The arrows indicate the number of genetic factors mapped to the segments shown below. The relative intensities of the genetic effect mapped to the segments are indicated as the gradation of the shades. The darker shades indicate segments with stronger effect. The data are from Ting et al. (2001).

carrying different lengths of Z chromosomal segments, they concluded that on the third chromosome, at least four loci contribute to the mating success of Z males. For the Z female mating preference, at least two (and more likely three) loci were inferred to be responsible. The locations of these chromosomal regions are shown in Figure 2.

In the accompanying analyses of the genetics of the second chromosome (Takahashi, Ting & Wu, in preparation), several modifications was made to improve the sensitivity of the assay. Recombinant lines free of visible markers were constructed, and an additional scheme for measuring weaker mate choice was used. In both chromosomes, there appears to be a pattern when testing the genetic effects on the segments in which the total effect is larger than the sum of the parts. This suggests that there may be interactions or epistasis among many of the detected loci such that the combinations of Z-alleles from two or more loci often have a synergistic effect. From these results, the genetic architecture underlying this behavioral polymorphism is with no doubt very complex.

Genetic mapping by genotype–phenotype association

An alternative approach to the deterministic genetic mapping above is the statistical approach using QTL mapping method. Recent QTL mapping studies on male reproductive traits show that multiple genes contribute to morphological divergence in sibling species of *Drosophila* (Liu et al., 1996; True et al., 1997; Macdonald & Goldstein, 1999; Zeng et al., 2000). For example, Zeng et al. (2000) showed evidence for 19 different QTL responsible for morphology of the male genital arch differences. In this Z/M system, none of the commonly used procedure such as the analysis of the F_2 backcross segregants is promising, because the trait value of the Z/M behavior is not designed to be measured from a single F_2 individual.

Instead of the conventional F_2-type QTL mapping approach, an analysis of the genotype–phenotype association has been tried out on the Z/M system. As in other QTL mapping studies, the key point of this method is to measure the correlation between genotype and phenotype at each marker position on the chromosome. The unique parts are the procedure in preparing the population for measuring association and that in measuring phenotypic trait. The method has been applied to the mapping of the Z male character on the third chromosome, and compared to the previous genetic mapping results obtained by constructing perpetual recombinant lines (Ting, Takahashi & Wu, 2001).

The first part of the method is to prepare a large population of individuals with finely recombined chromosomes of Z and M origins, whose genotype–phenotype association is to be measured. Figure 3 shows the schematic representation of the crosses performed to construct this hybrid population. The population was started out by crossing a Z strain that has inversion free third chromosome and an M strain with eight visible recessive markers on the third chromosome (*rucuca* line described in the previous section). After backcrossing 400 F_1s to *rucuca*, the population was cultured in a large population size (>2000 individuals) while waiting for the recombination to mix the two type chromosomes. At generation 7 (G_7), 300 individuals were backcrossed again to *rucuca.* At the following generation after this backcross (G_8), the flies that showed no marker phenotype were discarded. Since all the markers on *rucuca* are recessive, the individuals without any marker phenotype have a Z-type chromosome. This process at G_7 and G_8 was inserted to retain sufficient amount of M-type chromosomal segments in the population, which have a tendency to slowly decrease in frequency due to the defects of the marker phenotype. Then at generation 21 (G_{21}), the Z/M behavioral phenotype and the genotype of each

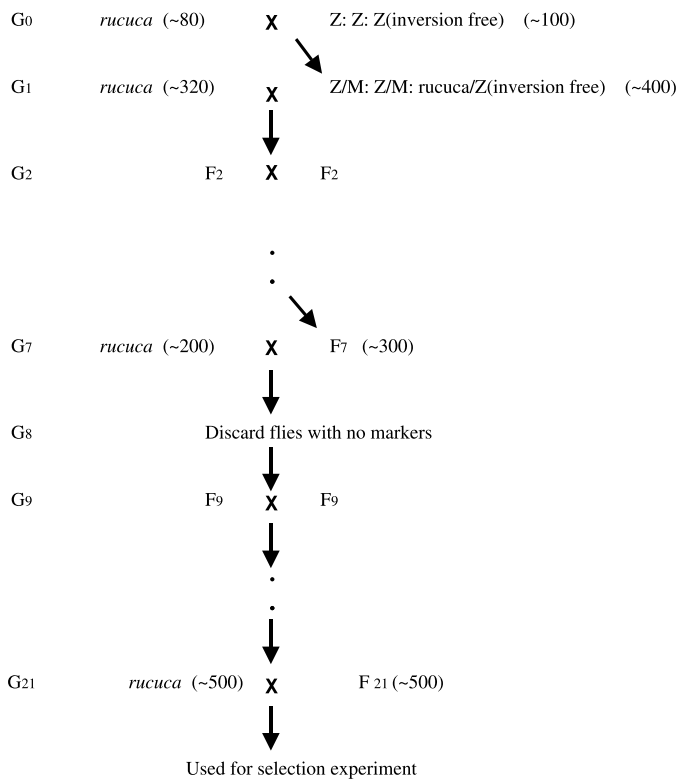

Figure 3. Schematic representation of the crosses performed to construct the Z/M hybrid population. The numbers in the parentheses indicate the number of flies used.

individual were assessed by a selection experiment described below.

At G_{21}, a sample of the population (∼500 males) was backcrossed to approximately 500 *rucuca* virgins to exhibit recessive markers that were visually hidden in heterozygous forms. Approximately 800 G_{22} males from this resulting backcross were subjected to the behavioral assay. The assay experiment was performed in four subsets and pooled for the later analyses with each set using approximately 200 G_{22} males, 100 Z30 (Z) females, and 200 Fr (M) females. They were released sequentially by the order described below into the mating cage after being fed by media containing different food colors for strain identification (see previous section).

The G_{22} test males were categorized into three groups according to their mating behavioral phenotype by the following scheme. First, the Z30 females and the test males were released into the cage. The Z-like group of experimental flies was selected by collecting the males that mated with Z30 females in the first 45 min. At this point, most of the Z-like males should have already mated. Then, Fr females were added. The small number of flies that mated with Z30 females after 45 min were added to the first category. The G_{22} males that mated with Fr females within 3 h from the start were those that were reluctant to mate with Z females but willing to mate with M females. Thus, they were identified as the M-like second group. The unmated flies left in the cages after 3 h were those that were not willing to mate with either Z or M females, and were categorized to the third group. Individuals in the third group and those in the other two groups that exhibited no markers were uninformative in terms of assessing the association between phenotype and genotype. Thus, they were excluded from the data analyses.

The first group consisted of 114 males in total (36 individuals were excluded). One hundred eighty-four males were classified into the second group (after excluding 48 males). The proportion of Z genotype individuals of the first and the second group at each marker are indicated according to its cytological position in Figure 4. One tail Fisher's exact test was performed at each marker to test for the independence of the Z/M phenotypic category (and the Z/M geno-

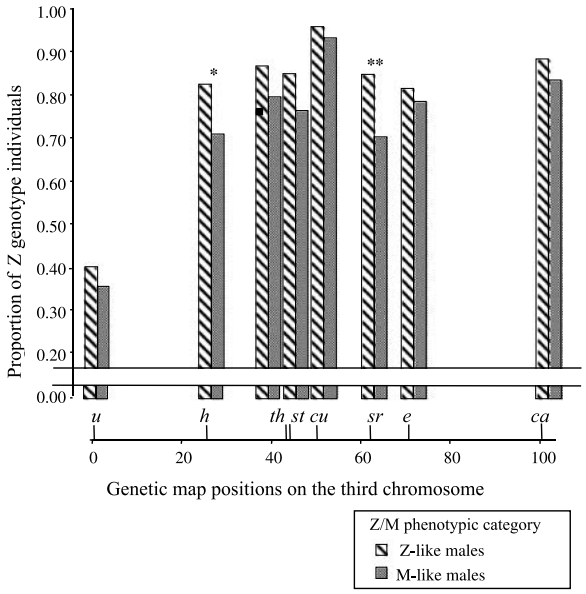

Figure 4. Results of the genotype–phenotype association mapping of the Z-maleness genes on the third chromosome. The proportion of the Z genotype individuals for each marker among the tested males in Z-like and M-like phenotypic categories are presented. Markers are indicated along the horizontal axis according to their genetic map positions. One tail Fisher's exact test was performed at each marker to test for the independence of the Z/M phenotypic category and the Z/M genotype. ** indicates uncorrected $P < 0.01$; $0.01 <$ Bonferroni corrected $P < 0.05$, and * indicates uncorrected $P < 0.05$; $0.05 <$ Bonferroni corrected $P < 0.10$.

type). The departure from independence was seen at two marker regions of the chromosome, the *h* region (uncorrected $P < 0.05$; $0.05 <$ Bonferroni corrected $P < 0.10$) and the *sr* region (uncorrected $P < 0.01$; $0.01 <$ Bonferroni corrected $P < 0.05$). We should note that the P value of the *h* region exceeded the 5% level after Bonferroni correction for multiple tests which is known to be overly conservative for most of the cases. The interpretation may require caution, however, here we treat the result as marginally significant for this case.

The genetic mapping results by chromosomal segment level comparisons revealed two segments, one surrounding *th* and *st* and another around *sr*, *e*, and *ca* markers, with strong Z-maleness effects, and one segment including *ru* and *h* with a weak effect (Ting, Takahashi & Wu, 2001; see previous section). From the genotype–phenotype association mapping, two marker regions on the third chromosome, the *h* and the *sr* regions, showed association with the male Z/M behavioral phenotype. These results obtained by two different means are roughly in agreement with each other.

The two strong genetic components revealed by the recombinant method were also detected in the association method. For the component in 3L region surrounding *th* and *st*, it is reasonable to presume that the effective region is in between *h* and *th* markers by the following interpretation, and thus came out as an association with the *h* marker in the latter method. The chromosomal segment recombinant method does not take into account where the exact breakpoints of the segments are. Whereas the association method is more sensitive to the distance between the genes and the markers. Therefore, if the effective region is closer to *h* than to *th*, there is a high chance that the former method would show effects on the segment surrounding either *h* or *th* and the latter method would show association with *h*, which is the case observed here.

For the 3R region, chromosomal segment comparison showed that there are at least two genes interacting with each other on the long segment including *sr*, *e*, and *ca*, but neither of the three small segments surrounding *sr*, *e*, or *ca* showed effect alone. (Ting, Takahashi & Wu, 2001; see previous section). By the association method, *sr* marker region showed significant association with the behavioral phenotype. Since the association mapping method can pick up genes with interactions as well as those act by themselves, the possible genetic structure could be that there is one major genetic component near the *sr* marker that interacts with other genes of small effects on the rest of the 3R region.

There were many sources of noise in this QTL mapping procedure. The X and the second chromosomes of the generated Z/M hybrid line had recombined and mixed as well as the third chromosome. In this experiment, we only tracked the frequency profile of Z and M fragments of the third chromosome. Although the third chromosome has stronger effect of Z behavioral character than X or the second chromosome (Hollocher et al., 1997b), the background noise could be created from the latter two chromosomes. Another source of noise could be from any defective effect caused by the mutant markers used for mapping. Higher resolution mapping by removing these sources should reveal more precise picture of the genetic architecture.

Moreover, the more sophisticated version of the mapping scheme recommended in Luo, Wu & Kearsey (2002) could be performed on this system. While waiting for the recombination to finely mix the genome

into fragments of Z and M chromosomes, one could apply selection for the strong Z character every certain number of generations, and also to backcross it with M line every certain number of generations. This will bring down the frequencies of Z chromosome fragments each time the population is backcrossed to M line leaving the fragments that carry Z genes which would be pulled up and maintained in high frequencies by the sequential selection.

The above recurrent selection and backcross (RSB) method has been suggested by Wright (1952), formalized by Hill (1998), and has been investigated in comparison with other QTL mapping schemes by Luo, Wu & Kearsey (2002). So far, there are limited applications to a real system (e.g., Beebe et al., 1997). Nevertheless, the method has advantage over conventional QTL mapping by F_2 backcrosses when the genetic architecture is complicated which is the case in the Z/M sexual isolation system. The preliminary results from our large scale experiment carried out here show promising outcome of the precise association mapping using this novel method.

Behavioral cues

The actual behavioral cues for the mate discrimination in this sexual isolation system have not yet being successfully identified. Our tentative view is that multiple cues are involved in the mating preferences that maybe redundant. So far, efforts to remove cues by physical manipulation such as to cut wings, glue antennas, or to shut out light have not revealed any significant key (Wu, personal communication). It is obvious that neither of these manipulations alone can completely block the means of mate choice in Z males and females.

Some differences in mating behavior have been observed by watching the mating pairs under the microscope. A male performs a courtship behavior towards a virgin female by following the female's abdomen while flipping its wings. Then at a certain point, mount on the female from the back by contacting its genitalia to that of the female. Overall, Z males' courtship tends to be more vigorous and aggressive compared to that of M males. For example, Z males curl the abdomen downwards and forwards while courting the female, whereas M males do not. Also Z males stay closer to females' abdomens and occasionally put their head under females' wings during the courtship. These differences were observed in several typical Z and M strains, however, comparisons among many lines are restricted by difficulty in quantification. The difference in male vigor could be affecting the mate choice, but the asymmetric mating pattern by Z and M females and males cannot be explained by mere difference in vigor. Thus, so far these anecdotal observations have not provided any information on an actual cue.

Another notable observation is in a behavior which males touch females' genitalia by their proboscis immediately before mounting. The length in millisecond between the touch and the mount is 5–10 times shorter in some Z lines than in most of the M lines (Takahashi & Wu, unpublished data). This timing seems to be robust within the isofemale line but the Z and M differences becomes vague after surveying more lines from the Z race. The similar picture has been obtained from the comparison of the interpulse interval (IPI) of the courtship songs between the two races (Colegrave et al., 2000). They observed that the Z flies have the shortest IPI, but no correlation with the assortative mating was indicated, suggesting little direct role of IPI in sexual isolation.

These results indicate that the different mating preference between the two behavioral races maybe defined by multiple redundant cues. A hypothetical view, schematically presented in Figure 5, can explain the complexity of the behavioral and the genetic aspects. Different behavioral loci could be fixed or segregating in different Z populations (Figure 5). Depending on the Z/M states at other loci, a locus may or may not correlate with the Z-ness in behavior produced by the effect of all the behavioral loci. For example, the Locus 1 in Figure 5 may have correlation with the Z/M behavior in population Z_1, but not in populations Z_2 and Z_3. In the latter two populations, polymorphisms at other loci contribute more to the behavior variation. One does not expect a strong correlation between a single behavioral trait and mating preference across populations in such a multi-locus system.

For example, *desat2* gene maybe one of such loci. Takahashi et al. (2001) identified a deletion polymorphism at the promoter region of this gene, which is responsible for the cuticular hydrocarbon polymorphism in *D. melanogaster* (Coyne, Wicker-Thomas & Jallon, 1999; Dallerac et al. 2001). This trait shows correlation with the Z/M behavior (Takahashi et al., 2001; Fang, Takahashi & Wu, 2002). However, African and Caribbean populations both have High type hydrocarbon, but Caribbean populations show M-type in behavior. Thus, the correlation is limited

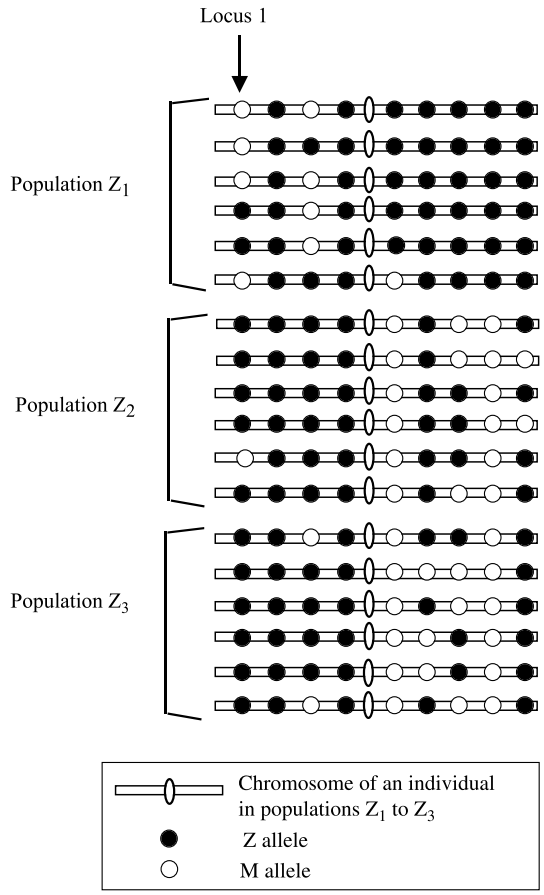

Figure 5. Hypothetical genetic structure of the Z loci in different Z populations. Circles indicate the position of Z/M behavioral loci on the chromosomes of individuals in different populations, Z_1–Z_3. Z and M alleles are represented as closed and open circles, respectively. The Z/M phenotype of the individual is determined by its genotype at all the behavioral loci. Note that different Z loci are fixed or segregating at different loci in different Z populations. For example, at Locus 1, Z allele is fixed in population Z_3, but segregating in populations Z_1 and Z_2.

to isofemale lines "within populations" in Africa. Probably, different Z/M behavioral loci, one of them possibly being the *desat2* locus, are either fixed or segregating in different Z populations.

Whether the cuticular hydrocarbon polymorphism is truly a determinant of the Z/M behavior differences or not remains to be tested. The exact mechanism for the correlation may not be very simple since the polymorphism is in female who appears to dominate the mate choice. It could be related to male choice, female response, and their synergism. Previously, Coyne and colleagues showed an effect of the cuticular hydrocarbon differences on male mate-choice between species of the *D. melanogaster* group (Coyne, Crittenden & Mah, 1994; Coyne & Charlesworth, 1997) but found no detectable effect on mating frequencies within *D. melanogaster* (Coyne, Wicker-Thomas & Jallon, 1999). Those experiments were done by rubbing off the cuticular hydrocarbon of a fly by crowding it into a vial with many flies with another type of hydrocarbon (details in Coyne & Charlesworth, 1997).

Identification and characterization of the behavioral loci in this system is an immediate task to be pursued. Nevertheless, it is possible that any genetic component to be found could only be detected via intrapopulation correlation.

Mating experiments against *Drosophila simulans*

An interesting trend has been observed in the premating isolation between *D. simulans* and the two *melanogaster* populations. A preliminary data shows that the isolation tends to be stronger between *D. simulans* and the Z population of *melanogaster* than between *D. simulans* and the M population (Sawamura & Wu, personal communication). It is possible that there maybe a correlation between the intensities of inter- and intraspecific sexual isolation in these sibling species.

Remaining questions

How different are the two races at the molecular level?

Although Ting, Takahashi and Wu (2001) on the Z character showed that there are multiple genes that are functionally divergent between the two races, how many genes have actually diverged, and what is the level of divergence between the two races remains to be pursued. The overall picture obtained from the genes surveyed throughout the genome shows that the African *D. melanogaster* populations do not seem to have largely differentiated from their worldwide counterparts (Aguade, 1998; Hasson et al., 1998; Tsaur, Ting & Wu, 1998; Andolfatto, 2001), except at some loci on the X chromosome (Begun & Aquadro, 1993). If one scans the genome of the individuals in a Z and an M population, the loci showing the fixed difference between the two maybe some of the behavior genes involved in sexual isolation. The fundamental unit of the process of speciation should be the gene, not the

individual or the whole genome, as is often implicitly assumed (Wu, 2001). The genomic scan of the two races, which may become feasible in this genomic era, should help identify loci pertaining to sexual isolation between these two behavior races.

In contrast to the differentiation at many loci in the Z/M system, there is a notable convincing case of surprisingly simple system revealed by Doi et al. (2001). Between *Drosophila ananassae* and *D. pallidosa*, a very small region near the marker, *Delta*, contributes disproportionately to females' preference for *D. ananassae* males (Doi et al., 2001). These species pairs seem to have diverged further than the Z/M behavioral races morphologically and genetically (Futch, 1966; Johnson et al., 1966; Hasson et al., 1998; Tsaur, Ting & Wu, 1998; Aguade, 1998; Andolfatto, 2001), although the number of genes diverged for the behavior isolation is smaller in the former pair. Whether the simple genetic architecture between the former species have allowed the ancestral populations to diverge quickly is an intriguing question (Butlin & Ritchie, 2001; Ting, Takahashi & Wu, 2001).

How is the Z/M polymorphism maintained?

Although we cannot predict the exact speed and direction of the evolutionary change that the sexual isolation system is undergoing, it is a prominent question to ask why the Z/M polymorphism has been persisting. If everything else is equal, the M-type individuals (males) should have mating disadvantage against Z-type counterparts in a polymorphic population by not being able to mate with the Z-type portion of the female population. However, Z-type does not seem to show the fate to go to fixation.

One explanation would be that there are other selective factors counteracting to balance the mating advantage of the Z-type. For example, individuals from Z populations are known to have lower fitness components when reared in a population cage in the laboratory compared to those from M populations (Alipaz, Karr, & Wu, in preparation). Or M phenotype may have some other ecological advantage in the field, which is not visible in the laboratory culture. There is very limited information about the ecological environment that they inhabit. Thus, slight differences in ecological factors could give different outcomes of competitive ability between the flies from the two races in the field.

Secondly, as discussed earlier, it is likely that speciation would be harder to achieve in general if reproductive isolation is based on a large number of loci that can potentially generate many intermediate phenotypes. In the Z/M system, it takes a large number of loci to render a high degree of sexual isolation between the strong Z-type and the cosmopolitan M-type. This may have resulted in a large number of intermediate phenotypes with many segregating loci (Hollocher et al., 1997b).

The third possibility is that the behavior genes are associated with certain chromosome rearrangements and are under some kind of balancing selection. There are many chromosome rearrangements reported in African populations (Krimbas & Powell, 1992). In particular, at least two (possibly more) genes with epistatic interactions responsible for male mating success were mapped into the region of *In(3R)K* (Ting, Takahashi & Wu, 2001; see previous section). This inversion is common in Africa but rare in other cosmopolitan populations (reviewed in Lemeunier & Aulard, 1992). However, by comparing the male mating success of *In(3R)K* homozygous line and standard 3R line both from Zimbabwe and Maryland (cosmopolitan) races, no strong association was found between this particular inversion and the Z/M phenotype (Takahashi & Wu, unpublished data). This is showing that despite the reduction of recombination around the breakpoints (Aquadro et al., 1991; Rozas et al., 1999), the inversion polymorphism may not be playing a role in the evolution of the complex trait such as the Z/M phenotype. This may be an important message for the implication of speciation by chromosome rearrangements.

Finally, Alipaz, Wu and Karr (2001) recently found a form of gametic incompatibility between the two races. The crosses between Z females and M males ($Z \times M$) produce far fewer offspring than reciprocal crosses due to a lower rate of egg hatch (range 39–85% in $Z \times M$ crosses versus range 79–94% in $M \times Z$ crosses). They observed large numbers of unfertilized and partially fertilized eggs in $Z \times M$ crosses, and thus concluded that egg inviability in these crosses was due to defects in fertilization. Whether this reduction in fitness contributes to any slight selective force of reinforcement to maintain this system is not certain at this point.

How has this system evolved?

From the molecular and biogeographical data (David & Capy, 1988; Lachaise et al., 1988; Singh & Long, 1992; Begun & Aquadro, 1992, 1995), the cosmopol-

itan populations are suggested to have recently derived from African populations. Z-type behavior may be representing the ancestral state from which the M-type behavior has evolved and subsequently spread throughout the world. The runaway process by sexual selection (Fisher, 1930; Lande, 1981; Kirkpatrick, 1982) expects the evolution towards more successful males with mating advantage, which seems to be in the opposite direction of the Z-type to M-type evolution (see discussion above).

Hollocher et al. (1997a) discuss this point from the following two models. One is the model by Kaneshiro (1983, 1989). In his model, the ancestral type female discriminates against the derived male through a relaxation of sexual selection brought on by founder effect (Kaneshiro, 1983, 1989). The behavior pattern predicted in the model is consistent with that in the Z/M system, however, this kind of severe bottleneck is unlikely to have taken place in this species (more discussion in Hollocher et al., 1997a).

The second model is that on runaway process by Iwasa and Pomiankowski (1995). Hollocher et al. (1997b) explains as the following. The mathematical model predicts that the self-reinforcing 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 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 may account for why derived populations often show relaxed female preference in mating. They bring up the possibility that the current populations in African continent may be representing various stages of the sliding-back phase, regressing toward the M-state (more discussion in Hollocher et al., 1997b). The application of this particular model seems rather too specific in explaining the behavioral polymorphism. Nevertheless, sexual selection is probably playing a key role in this system and awaits further investigation at the molecular level.

Conclusions

We have documented the complex genetic basis and behavioral features of the Z/M sexual isolation system. The genetics underlying the traits involved in speciation could be either simple or complex (see Table 1 of Coyne & Orr, 1998), and there are still not enough comparable cases to find a general rule to this volume. However, our motto is that we should not shy away from studying the complex systems when we happen to face one. Thus, we have reviewed the studies aimed to understand the complex genetic basis underlying this system. The resolution so far is at the chromosomal segment level. Several more rounds of intensive effort need to be put in before identifying the behavioral genes. Mapping by phenotype–genotype association maybe a hint to an application of a new method.

As has been demonstrated by the successful case of *Odysseus* (Ting et al., 1998; Ting, Tsaur & Wu, 2000), further insights into the underlying evolutionary mechanism of this putative speciation event awaits the cloning and the sequence level molecular evolutionary analyses on the behavioral loci.

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