Evolution, 43(6), 1989, pp. 1308–1311

NOTES AND COMMENTS

REPRODUCTIVE ISOLATION AS A CONSEQUENCE OF ADAPTIVE DIVERGENCE IN *DROSOPHILA PSEUDOOBSCURA*

DIANE M. B. DODD[1]
Department of Biology, Yale University, New Haven, CT

Received May 27, 1988. Accepted March 31, 1989

According to the biological species concept, speciation is basically a problem of reproductive isolation. Of the many ways to classify isolating mechanisms, the two main divisions are premating isolation, in which mating is prevented from occurring, and postmating isolation, in which mating takes place but viable, fertile offspring are not produced. There is much debate over which type of mechanism, premating or postmating, is most likely to develop first and how the isolation comes about (e.g., see Dobzhansky, 1970; Mayr, 1963; and Muller, 1949).

In an attempt to gain insight into the process of the development of reproductive isolation, eight populations of *Drosophila pseudoobscura* were studied. These were first used by Powell and Andjelkovič (1983) in a study of the alpha-amylase (*Amy*) locus. Four were reared on a starch-based medium, and four were reared on a maltose-based medium. These two media are both quite stressful; it initially took several months for the populations to become fully established and healthy. Considering the pressure placed on the populations by the media, one would expect to see some kind of adaptive divergence between the starch-reared and maltose-reared flies.

Several changes were in fact observed in the eight populations. Powell and Andjelkovič noted an increase in the "*fast*" allele of *Amy* in the starch populations as well as an increase in one of the patterns of amylase activity in the midgut. However, no corresponding changes were seen in the maltose populations. Elsewhere (Dodd, 1984), I have presented evidence that the populations have become differentially adapted to the two media. In this study, it is shown that the populations have also developed behavioral isolation as a pleiotropic by-product of this adaptive divergence.

MATERIALS AND METHODS

All eight *D. pseudoobscura* populations were derived from a single population collected at Bryce Canyon, Utah (see Powell and Andjelkovič [1983] for details on the media and the generation of the populations). The four starch-reared populations were designated Ist–IVst; the maltose-reared populations were designated Ima–IVma. The flies were maintained in population cages at 25°C. The present investigation was begun approximately one year after the populations were started.

Starch-adapted populations were tested against maltose-adapted populations in every possible combination to determine whether adaptation to the two new regimes could have induced the development of ethological isolation. Multiple-choice tests were performed using mating chambers modeled on those described by Elens and Wattiaux (1964). All flies used in the mating-preference tests were reared for one generation on standard cornmeal-molasses-agar medium. Virgin males and females were anesthetized with CO_2, isolated from the opposite sex, and aged on standard medium for 3–6 days. Twelve females from each of the populations to be tested were placed in the chamber. Twelve males from the two populations were then introduced as nearly simultaneously as possible. The flies were not anesthetized for this procedure. The tests were performed at room temperature (no higher than 25°C), under bright (but not direct) lighting. The chambers were observed for 60–90 minutes.

Individuals of one population had the tips of their right wings clipped to allow identification. At least two replicates of each test were performed, with the wing clipping alternated between populations. Wing clipping has not been found to interfere with mating success in *Drosophila* (Ehrman, 1966; Ehrman and Petit, 1968; Powell, 1978; Robertson, 1982; Knoppien, 1984; van den Berg et al., 1984; Dodd and Powell, 1985; Spiess, 1986), and once again in the present tests, wing clipping had no effect on mating propensity in either sex. Of the 1,558 matings scored, 778 were with nonclipped males, and 780 were with clipped males; 793 nonclipped females mated, while 765 clipped females mated. These differences are not statistically significant.

An isolation index (I) was calculated for each mating test. The index used follows Stalker (1942), Bateman (1949), and Merrell (1950), with the standard error derived following Malogolowkin-Cohen et al. (1965):

$$I = \frac{\text{homogamic matings} - \text{heterogamic matings}}{\text{total matings } (N)}$$

$$\text{SE of } I = \sqrt{(1 - I^2)/N}\,.$$

I ranges from -1 to 1; a value of zero indicates random mating; $I > 0$ indicates positive assortative mating; and $I < 0$ indicates negative assortative mating. Contingency chi-square tests were also performed to check for deviations from random mating.

[1] Present address: Department of Biological Sciences, University of North Carolina at Wilmington, Wilmington, NC 28403-3297.

Results

The results of the mating-preference tests between starch-adapted and maltose-adapted populations are given in Table 1. Contingency chi-square tests reveal that 11 out of the 16 combinations show significant deviation from expectations based on random mating. The isolation indexes of these crosses all indicate positive assortative mating, ranging from 0.30 ± 0.13 to 0.49 ± 0.10. The crosses that do not show significant departure from random mating also have positive isolation indexes, ranging from 0.18 ± 0.14 to 0.24 ± 0.13. A one-tailed sign test (Champion, 1981 pp. 276–280) on the indexes shows that the probability of obtaining 16 positive indexes for 16 crosses is less than 0.001.

It is possible that the behavioral isolation apparent between the starch- and maltose-adapted populations is a result of bottlenecks in population size when the populations were first established. Thus, the populations may have diverged due to such founder-flush effects as proposed by Carson (1971, 1975) and experimentally observed by Powell (1978). If this were the case, there should be isolation between populations within the same regime. Therefore, each starch-adapted population was tested with each other starch-adapted population. Likewise, each maltose-adapted population was tested with each other maltose-adapted population.

Table 2 gives the data for these crosses. None of the within-regime tests deviates significantly from expectations for random mating according to the X^2 tests. The indexes range from −0.06 ± 0.14 to 0.15 ± 0.13 in the starch-regime tests and from −0.21 ± 0.13 to 0.18 ± 0.14 in the maltose-regime tests. Four of the six within-maltose-regime crosses show heterogamic preferences, as do two of the six within-starch-regime crosses. Two-tailed sign tests were again performed to confirm the randomness of the indexes: starch regime: $P = 0.688$; maltose regime: $P = 0.688$; all within-regime crosses: $P > 0.999$.

There is no assortative mating within regimes. Averaging the isolation indices within the three categories illustrates the general pattern: 0.33 for the tests of starch versus maltose, 0.05 for the tests within the starch regime, and −0.01 for the tests within the maltose regime.

Discussion

Significant behavioral isolation between starch-adapted and maltose-adapted populations was observed. The isolation was not a result of conditioning of the flies to the two media, since all tests were performed using flies that had been reared on a common medium and had experienced neither starch nor maltose. Nor was physical isolation alone responsible for the changes in mating behavior, since there was no evidence of behavioral isolation between any pair of the four starch-adapted populations nor between any pair of the four maltose-adapted populations.

The ethological isolation was a pleiotropic by-product of the adaption of the populations to the two media, confirming one of the basic tenets of the Modern Synthesis. Reproductive isolation was not the target of the selection, and there was no a priori reason to believe

TABLE 1. Results of mating-preference tests between starch-adapted and maltose-adapted populations. I = isolation index (see text). The results of contingency chi-square tests for each cross are also given.

A.

Males	Females Ist	Ima
Ist	18	11
Ima	10	12

$I = 0.18 \pm 0.14$
$X^2 = 1.39$

B.

Males	Females Ist	IIma
Ist	22	9
IIma	8	20

$I = 0.42 \pm 0.14$
$X^2 = 10.58^{**}$

C.

Males	Females Ist	IIIma
Ist	17	5
IIIma	12	19

$I = 0.36 \pm 0.13$
$X^2 = 7.72^{**}$

D.

Males	Females Ist	IVma
Ist	16	8
IVma	7	25

$I = 0.46 \pm 0.12$
$X^2 = 11.37^{***}$

E.

Males	Females IIst	Ima
IIst	14	12
Ima	6	22

$I = 0.33 \pm 0.13$
$X^2 = 6.07^{*}$

F.

Males	Females IIst	IIma
IIst	19	7
IIma	8	22

$I = 0.46 \pm 0.12$
$X^2 = 12.02^{***}$

G.

Males	Females IIst	IIIma
IIst	17	8
IIIma	11	19

$I = 0.31 \pm 0.13$
$X^2 = 5.36^{*}$

H.

Males	Females IIst	IVma
IIst	23	11
IVma	8	16

$I = 0.35 \pm 0.12$
$X^2 = 6.66^{**}$

I.

Males	Females IIIst	Ima
IIIst	16	12
Ima	9	17

$I = 0.22 \pm 0.13$
$X^2 = 2.56$

J.

Males	Females IIIst	IIma
IIIst	18	11
IIma	10	16

$I = 0.24 \pm 0.13$
$X^2 = 3.06$

K.

Males	Females IIIst	IIIma
IIIst	26	10
IIIma	9	29

$I = 0.49 \pm 0.10$
$X^2 = 17.48^{***}$

L.

Males	Females IIIst	IVma
IIIst	16	12
IVma	10	17

$I = 0.20 \pm 0.13$
$X^2 = 2.23$

M.

Males	Females IVst	Ima
IVst	17	11
Ima	9	20

$I = 0.30 \pm 0.13$
$X^2 = 5.06^{*}$

N.

Males	Females IVst	IIma
IVst	18	5
IIma	12	19

$I = 0.37 \pm 0.13$
$X^2 = 8.37^{**}$

O.

Males	Females IVst	IIIma
IVst	17	11
IIIma	9	21

$I = 0.31 \pm 0.12$
$X^2 = 5.52^{*}$

P.

Males	Females IVst	IVma
IVst	16	10
IVma	11	18

$I = 0.24 \pm 0.13$
$X^2 = 3.06$

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Mating-preference tests within regimes. Each starch-adapted population was tested against each other starch-adapted population, and each maltose-adapted population was tested against each other maltose-adapted population. I (isolation index) and contingency X^2 are given for each cross.

A.	Females	
Males	Ist	IIst
Ist	18	15
IIst	12	15

$I = 0.10 \pm 0.13$
$X^2 = 0.40$

B.	Females	
Males	Ist	IIIst
Ist	13	9
IIIst	16	19

$I = 0.12 \pm 0.13$
$X^2 = 0.97$

C.	Females	
Males	Ist	IVst
Ist	13	11
IVst	11	17

$I = 0.15 \pm 0.13$
$X^2 = 1.15$

D.	Females	
Males	IIst	IIIst
IIst	12	11
IIIst	16	12

$I = -0.04 \pm 0.14$
$X^2 = 0.05$

E.	Females	
Males	IIst	IVst
IIst	15	12
IVst	14	12

$I = 0.02 \pm 0.14$
$X^2 = 0.02$

F.	Females	
Males	IIIst	IVst
IIIst	16	12
IVst	16	9

$I = -0.06 \pm 0.14$
$X^2 = 0.26$

G.	Females	
Males	Ima	IIma
Ima	17	14
IIma	10	17

$I = 0.17 \pm 0.13$
$X^2 = 1.84$

H.	Females	
Males	Ima	IIIma
Ima	14	9
IIIma	12	16

$I = 0.18 \pm 0.14$
$X^2 = 1.64$

I.	Females	
Males	Ima	IVma
Ima	12	12
IVma	15	14

$I = -0.02 \pm 0.14$
$X^2 = 0.02$

J.	Females	
Males	IIma	IIIma
IIma	11	14
IIIma	18	10

$I = -0.21 \pm 0.13$
$X^2 = 0.82$

K.	Females	
Males	IIma	IVma
IIma	12	14
IVma	14	14

$I = -0.04 \pm 0.14$
$X^2 = 0.08$

L.	Females	
Males	IIIma	IVma
IIIma	11	18
IVma	15	14

$I = -0.14 \pm 0.13$
$X^2 = 1.12$

that adaptation to starch or maltose should have any effect on mating behavior, yet isolation developed.

Similar studies resulting in the development of stable premating isolation due to adaptive divergence have been reported. Kilias et al. (1980) observed behavioral isolation between strains of *D. melanogaster* reared at different temperatures and relative humidities. Flies from the same base population subjected to different regimes developed reproductive isolation, while flies from different gene pools reared under the same conditions exhibited random mating. Markow (1981) selected for phototactic and geotactic behavior in *D. melanogaster*. Behavioral isolation was evident between some pairs of the selected and control populations. Soans et al. (1974) and Hurd and Eisenberg (1975) reported reproductive isolation in housefly (*Musca domestica*) populations selected for positive and negative geotaxis.

The results of this study also demonstrate that reinforcement of premating isolating mechanisms through selection is not necessary for the development of significant levels of behavioral isolation. The isolation observed here developed in complete allopatry. The populations were maintained separately at all times, and thus there was no opportunity for reinforcement through selection against hybrids. The isolation is due solely to the process of adaptation to the novel regimes.

This process led to consistent changes in all four populations under each regime. Each of the four populations subjected to the same regime acquired the same (or similar) changes in mating behavior, such that flies from different populations under the same regime are not isolated. Isolation is only evident between regimes.

The mechanism of the isolation in this system is as yet unknown. Kilias et al. (1980) noted for one of their nine combinations that females adapted to one regime (cool, dry) mated more frequently than females from the second regime (warm, humid). Yet in another case, males reared in the warm, humid regime were more active than the cool-adapted males. Overall, there was no significant difference in sexual activity, as measured by numbers of each type mating, in either sex. Similarly, in this study chi-square tests revealed no significant differences in the numbers of flies from each population involved in matings ($X^2_{\text{males}} = 4.5$, $X^2_{\text{females}} = 1.8$, $d.f. = 7$). There is no difference in sexual activity between flies from the two regimes. Possible differences in specific courtship behaviors are presently being examined.

ACKNOWLEDGMENTS

I thank J. R. Powell, C. S. Dunn, and anonymous reviewers for providing critical reviews of this manuscript. Support was provided by NIH National Research Service Award #T32 GM07499-06 to D.M.B.D. and NSF grant DEB-8203119 to J. R. Powell.

LITERATURE CITED

BATEMAN, A. J. 1949. Analysis of data on sexual isolation. Evolution 3:174–177.

CARSON, H. L. 1971. Speciation and the founder principle. Stadler Symp. 3:51–70.

———. 1975. The genetics of speciation at the diploid level. Amer. Natur. 109:83–92.

CHAMPION, D. J. 1981. Basic Statistics for Social Research. Macmillan, N.Y.

DOBZHANSKY, TH. 1970. Genetics Of The Evolutionary Process. Columbia Univ. Press, N.Y.

DODD, D. M. B. 1984. Behavioral correlates of the adaptive divergence of *Drosophila* populations. Ph.D. Diss. Yale Univ., New Haven, CT.

DODD, D. M. B., AND J. R. POWELL. 1985. Founder-

flush speciation: An update of experimental results with *Drosophila.* Evolution 39:1388–1392.

EHRMAN, L. 1966. Mating success and genotype frequency in *Drosophila.* Anim. Behav. 14:332–339.

EHRMAN, L., AND C. PETIT. 1968. Genotype frequency and mating success in the *willistoni* species group of *Drosophila.* Evolution 22:649–658.

ELENS, A. A., AND J. M. WATTIAUX. 1964. Direct observation of sexual isolation. Dros. Inf. Serv. 39: 118–119.

HURD, L. E., AND R. M. EISENBERG. 1975. Divergent selection for geotactic response and evolution of reproductive isolation in sympatric and allopatric populations of houseflies. Amer. Natur. 109:353–358.

KILIAS, G., S. N. ALAHIOTIS, AND M. PELECANOS. 1980. A multifactorial genetic investigation of speciation theory using *Drosophila melanogaster.* Evolution 34:730–737.

KNOPPIEN, P. 1984. The rare male mating advantage: An artifact caused by marking procedures? Amer. Natur. 123:862–866.

MALOGOLOWKIN-COHEN, CH., A. SOLIMA SIMMONS, AND H. LEVENE. 1965. A study of sexual isolation between certain strains of *Drosophila paulistorum.* Evolution 19:95–103.

MARKOW, T. A. 1981. Mating preferences are not predictive of the direction of evolution in experimental populations of *Drosophila.* Science 213: 1405–1407.

MAYR, E. 1963. Animal Species And Evolution. Belknap, Cambridge, MA.

MERRELL, D. J. 1950. Measurement of sexual isolation and selective mating. Evolution 4:326–331.

MULLER, H. J. 1949. The Darwinian and modern conceptions of natural selection. Proc. Amer. Phil. Soc. 93:459–470.

POWELL, J. R. 1978. The founder-flush speciation theory: An experimental approach. Evolution 32: 465–474.

POWELL, J. R., AND M. ANDJELKOVIČ. 1983. Population genetics of Drosophila amylase. IV. Selection in laboratory populations maintained on different carbohydrates. Genetics 103:675–689.

ROBERTSON, H. M. 1982. Female courtship summation in *Drosophila melanogaster.* Anim. Behav. 30:1105–1117.

SOANS, A. B., D. PIMENTEL, AND J. S. SOANS. 1974. Evolution of reproductive isolation in allopatric and sympatric populations. Amer. Natur. 108:117–124.

SPIESS, E. B. 1986. Discrimination among prospective mates in *Drosophila,* pp. 75–119. *In* D. J. C. Fletcher and C. D. Michener (eds.), Kin Recognition in Animals. Wiley, N.Y.

STALKER, H. D. 1942. Sexual isolation studies in the species complex *Drosophila virilis.* Genetics 27:238–257.

VAN DEN BERG, M. J., G. THOMAS, H. HENDRIKS, AND W. VAN DELDEN. 1984. A reexamination of the negative assortative mating phenomenon and its underlying mechanism in *Drosophila melanogaster.* Behav. Genet. 14:45–61.

Corresponding Editor: J. A. Coyne

Evolution, 43(6), 1989, pp. 1311–1315

FEMALE RECEPTIVITY TO REMATING AND EARLY FECUNDITY IN *DROSOPHILA MELANOGASTER*

H. HENRY FUKUI AND MARK H. GROMKO
Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403

Received May 27, 1988. Accepted March 31, 1989

In *Drosophila melanogaster,* female attractiveness and receptivity is reduced following mating, and there seem to be two causes for these changes. There is a short-term effect of copulation which diminishes within 24 hours (Manning, 1967; Scott, 1987). Seminal-fluid components influence the effectiveness of this short-term reduction of female receptivity (Scott, 1987; Chen et al., 1988). The second effect, which was identified by Manning (1962, 1967) as a "sperm effect," is longer-lasting and decreases as the number of sperm in storage diminishes.

Studies on established lab stocks of *D. melanogaster* demonstrate that: 1) the return of receptivity in mated females is influenced by the number of sperm in storage (Manning, 1962, 1967; Gromko and Pyle, 1978; Gilbert et al., 1981; Gromko et al., 1984; Letsinger and Gromko, 1985); 2) experimental design influences the expression of the sperm effect (Gromko and Pyle, 1978; Newport and Gromko, 1984); 3) the strength of the sperm effect can be modified by selection (Gromko and Newport, 1988*a*); and 4) environmental factors influence the frequency of remating, with remating frequency being greatly reduced in the absence of food (Harshman et al., 1988).

Most work on these phenomena has been carried out with established laboratory stocks. A previous study indicated that remating frequency changed substantially in a population maintained in a large population cage (Gromko and Newport, 1988*a*). Thus, in the present study, we assayed fecundity and receptivity to remating in three populations derived recently from the field. We also compared four stock-maintenance pro-