Ethological Isolation Among Three Species of the *planitibia* Subgroup of Hawaiian *Drosophila*

(mating behavior/hybrid sterility/speciation)

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ABSTRACT *Drosophila heteroneura* and *D. silvestris* are sympatric species living on the island of Hawaii, while *D. planitibia* is allopatric on the nearby island of Maui. A pronounced ethological isolation is found between the sympatric species, and none between allopatric ones, except that *D. planitibia* females discriminate against *D. heteroneura* males. Male hybrids are sterile in allopatric crosses but fertile in sympatric ones. The ethological isolation and the hybrid sterility are uncorrelated. This is expected if premating isolation between closely related species is an *ad hoc* product of natural selection, while postmating isolation is an incidental result of genetic divergence. Some exceptions to the rule are discussed.

The archipelago of Hawaii, with total area somewhat smaller than that of the state of New Jersey, has at least 306 endemic species of Drosophila, about 25% of a world fauna of perhaps 1250 species (1). Since the geological age of the islands varies from about six to less than one million years, the proliferation of Drosophila species in Hawaii is relatively recent and is presumably still going forward. Several groups of Hawaiian endemics are very favorable materials for genetic and cytological studies of the speciation process. One of these is a cluster of three closely related but morphologically distinguishable species. *Drosophila heteroneura* (Perkins) and *D. silvestris* (Perkins) are both found on the largest and geologically youngest island, Hawaii, where they occur together in a number of localities. *D. planitibia* (Hardy) lives on the geologically older island of Maui (which is by no means the oldest in the archipelago).

The close relationship of the above species is attested by their chromosomal similarity. Not only are the metaphase chromosomes identical, but so are the gene arrangements, as inferred from similar disc patterns in the giant chromosomes of the larval salivary glands (2). There are, however, chromosomal inversion polymorphisms in two of the species—*D. heteroneura* and *D. silvestris*. *D. planitibia* is chromosomally monomorphic. In *D. heteroneura* there has been found a single inversion, which is quite common in some populations. (The inverted sequence has a frequency of about 60% on the Kona Coast of Hawaii.) *D. silvestris* is highly polymorphic, inversions being found in at least three of the chromosomes (the second, third, and fourth). Nevertheless, some individuals have the gene arrangements in all chromosomes identical with those in *D. planitibia* and *D. heteroneura*. As shown below, some interspecific crosses do not easily succeed owing to ethological (sexual) isolation. F_1 hybrids have been obtained in laboratory experiments (3). Those of *D. planitibia* with

either *D. silvestris* or *D. heteroneura* give fertile F_1 females but sterile males. Crosses of the sympatric species *D. silvestris* ♀ × *D. heteroneura* ♂ give fertile hybrids of both sexes. Recently, several successful crosses of the reciprocal combination have been obtained. These yielded abundant F_1 hybrids, which are also fertile in both sexes. Details of the hybridization experiments will be published elsewhere. Two problems are at issue in the present investigation. First, is the ethological isolation stronger between sympatric than between allopatric species? Second, are ethological isolation and hybrid sterility correlated?

MATERIALS AND METHODS

Laboratory strains of the three species were derived from inseminated females collected on Maui (*D. planitibia*) and on Hawaii (*D. silvestris* and *D. heteroneura*). These are maintained at a temperature of 18° on nutrient medium devised by Wheeler and Clayton (4). The flies to be used in the experiments were aged, the two sexes separately, until they were 30 days old. One female of each of two species was placed with one male of one of these or a third species in a 12-dram vial with food for 1 or 2 weeks. The females were then dissected and the presence or absence of sperm in the seminal receptable and spermathecae was diagnosed under a microscope (magnification approximately 100 times). This technique is known, rather misleadingly, as the "male choice method." Actually the choice is exercised mainly or only by the females who accept or reject the males courting them (5).

RESULTS

Experiments in which females of two species were placed with males of one of them are reported in Table 1. The sympatric species, *D. heteroneura* and *D. silvestris*, show a pronounced ethological isolation. Stalker's isolation indices (6) are almost identical, 0.68 and 0.70. An index of unity would mean that no heterogamic matings occur, while an index of zero would indicate that the two species mate at random. Lack of ethological isolation is observed with the allopatric *D. silvestris* and *D. planitibia*. Homogamic and heterogamic matings are nearly equally frequent; the chi squares of 0.39 and 1.21 have probabilities of chance occurrence of about 0.5 and 0.3, respectively. The deviation from randomness of mating is statistically not significant. With the other pair of allopatric species, *D. planitibia* and *D. heteroneura*, a quite interesting situation is found. *D. planitibia* males, which do not occur on the island of Hawaii, are not discriminated against by *D.*

TABLE 1. *Numbers of females tested, and of females inseminated, in experiments in which single females of each of two species were placed with a single male of one of them*

| Fe-males* | Male* | Homogamic | | Heterogamic | | I† | Chi square‡ |
		Test-ed	In-semi-nated	Test-ed	In-semi-nated		
h + s	h	27	21	27	4	0.68	19.1
h + s	s	26	18	26	3	0.70	15.7
s + p	s	32	11	33	8	0.17	0.39
s + p	p	32	21	32	16	0.14	1.21
h + p	p	28	11	28	10	0.05	0.0
h + p	h	29	21	30	4	0.69	18.7

* h = *Drosophila heteroneura*, s = *D. silvestris*, p = *D. planitibia*.

† Stalker's isolation index.

‡ Calculated from a 2 × 2 contingency table by use of the Yates correction.

heteroneura females, which are native there. In contrast to this, *D. heteroneura* males rarely succeed in mating with *D. planitibia* females. It is as if *D. heteroneura* males have evolved a specific odor or a courtship ritual that females of other species find unacceptable.

In the experiments reported in Table 2, one female of each of two species was exposed to a male of a third species. Thus, the female had only a choice of accepting a male of a foreign species or not mating at all. Indeed, most females remained virgin after 2-weeks' exposure to the males. Only small numbers of females were inseminated, and this makes conclusions as to the mating preferences of these females rather hazardous. Taking the data at face value, *D. silvestris* and *D. heteroneura* males were equally successful (or rather unsuccessful) with females of both species with which they were placed. The chi squares are much below the conventional level of statistical significance. Note however that in these tests one of the females belonged to a species sympatric, and the other allopatric, to the male. The situation is different with *D. planitibia* males. When placed with females of two allopatric species, they were accepted more often by *D. silvestris* than by *D. heteroneura* females.

TABLE 2. *Numbers of females tested, and of those found inseminated, in experiments in which single females of each of two species were placed with a single male of a third species*

Females	Male	Tested	Insemi-nated	Chi square
h + p	s	h 23	7	0.48
		p 23	4	
s + p	h	s 29	5	0.40
		p 29	8	
s + h	p	s 27	15	8.12
		h 27	4	

Abbreviations as in Table 1.

DISCUSSION

Two kinds of reproductive isolating mechanisms are found among the three species used in our experiments: ethological isolation and sterility of hybrid males. The former is a premating and the latter a postmating isolating barrier. Reproductive isolation may arise as a by-product of genetic divergence of incipient species, or it may be induced by natural selection as a barrier to gene exchange, if the latter is detrimental to the species concerned. Premating isolation may therefore be expected to occur more often between sympatric than between allopatric species. Postmating isolation may or may not be related to the geographic situation. The data reported in the present communication conform in part, but only in part, to the expectations derived from the above hypothesis. Pronounced ethological isolation is found between the sympatric *D. heteroneura* and *D. silvestris*. To be sure, it is incomplete, at least under laboratory conditions. It may be complete in nature—this is so, for example, between the sibling species *D. pseudoobscura* and *D. persimilis*, which produce hybrids in experiments but do so extremely rarely in nature (7). Ethological isolation is found neither between the allopatric *D. planitibia* and *D. silvestris* nor between *D. planitibia* and *D. heteroneura* when *D. planitibia* is the male. *D. heteroneura* males are nevertheless rather seldom accepted by the allopatric *D. planitibia* females. Experiments with females of two species exposed to males of a third species give results that are difficult to interpret. *D. silvestris* males seem to be discriminated against about equally by the sympatric *D. heteroneura* and the allopatric *D. planitibia* females. The same is true of *D. heteroneura* males confronting *D. planitibia* and *D. silvestris* females, and yet *D. planitibia* males are more successful with the allopatric *D. silvestris* than with the likewise allopatric *D. heteroneura* females.

The island of Hawaii, inhabited by *D. silvestris* and *D. heteroneura*, is geologically younger than adjacent Maui and the other islands lying to the northwest. A proposal has been made (8) that *D. silvestris* and *D. heteroneura* arose by speciation on the large island of Hawaii following a single colonization from Maui. A second possibility is that these two species arose from two separate colonizations of Hawaii. These could have come from Maui or, indeed, from planitibia-like ancestors on Molokai or even Oahu, further to the northwest. In either case, the two sympatric species on Hawaii would be expected to build up a premating isolating mechanism, such as ethological isolation. This is what is actually observed. There is no necessity for *D. planitibia* to be ethologically isolated from the species living on Hawaii. There is no isolation between *D. planitibia* from Maui and *D. silvestris* from Hawaii. Yet *D. heteroneura* males from Hawaii are discriminated against by Maui *D. planitibia*. Further light on this situation will very likely come from clarification of the genetic status of planitibia-like flies on Maui and also from the adjacent island of Molokai. Newly obtained strains of planitibia subgroup flies from Molokai are now under investigation. It is even possible, though much less likely, that *D. planitibia* on Maui arose through return colonization of that island by a silvestris-like form. Ethological isolation between allopatric forms may be a vestige of their evolutionary history.

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