# ETHOLOGICAL ISOLATION AND PHYLOGENY
## IN THE *PLANITIBIA* SUBGROUP
## OF HAWAIIAN *DROSOPHILA*[1]

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Spieth (1966, 1968*a*, 1968*b*) reported laboratory and field observations of the bizarre courtship behavior patterns displayed by Hawaiian drosophilids and suggested the role of ethological isolation as one of the major reproductive isolating mechanisms in the endemic fauna. Additionally, Spieth (1966) showed that qualitative analyses of these courtship patterns permit one to infer the phylogenetic relationships of species sharing elements of these patterns. Although preliminary observations of courtship patterns of the four species studied in this paper have been recorded by Spieth (pers. comm.), detailed analyses of these patterns have not been reported. Nevertheless, the data presented in this paper, measuring the amount of premating reproductive isolation among the four species in "male-choice" experiments, provide basis for the interpretation of phylogenetic relationships of these species.

The subjects of this study are four closely related picture-winged species of Hawaiian *Drosophila*. *Drosophila differens* and *planitibia* inhabit the rain forests of Molokai and Maui respectively, while *heteroneura* and *silvestris* are sympatric over a wide range on the Big Island of Hawaii. The chromosomal relationships of all four species have been reported by Carson and Stalker (1968) and Craddock (1974). *D. differens* and *planitibia* are monomorphic and homosequential in banding patterns of polytene chromosomes while *heteroneura* and *silvestris* share a

unique polymorphic inversion in chromosome 3 (3m/+). In addition, *silvestris* carries seven other polymorphic inversions not found in the other three species. Thus, there are no fixed inversion differences in the karyotype of the four species. Equally intriguing results have been obtained by measurements of genetic similarity (Johnson et al., 1975). Analyses of allozymic variation and calculation of similarity indices among the four species revealed an unusually high level of genetic similarity ranging from 0.71–0.96 (Rogers' similarity coefficient, 1972).

Ahearn et al. (1974) studied the degree of ethological isolation which exists between three of the species and postulated that a definitive study of the ethological relationship of *differens* to the other three species would further elucidate the speciation mechanisms in the evolution of the two species found on the island of Hawaii, i.e. *heteroneura* and *silvestris*. The data presented in this study provide an indication of the degree of ethological isolation which might exist between any two of these four species. To be sure, behavorial studies under laboratory conditions may present a biased account of the natural situation. Nevertheless, with properly controlled experiments, it may be possible to extrapolate from the data the biological and phylogenetic significance of various behavorial patterns.

## MATERIALS AND METHODS

Virgin females and males were collected from laboratory strains of *planitibia* (M82C4—Waikamoi, Maui), *differens* (R83B17—South Hanalilolilo, Molokai), *heteroneura* (Q71G12—Olaa Forest Re-

serve, Hawaii), and *silvestris* (R59G4— Kilauea Forest Reserve, Hawaii). The flies were aged, the two sexes separately, on Wheeler-Clayton medium (Wheeler and Clayton 1965) at a temperature of 18– 19C until they were about four weeks old. This seemingly long maturation period of four weeks was necessary since preliminary observations indicated that males were not able to perform effective courtship rituals until they were 15–16 days old (Ahearn, pers. comm.). After the maturation period, a single female of each of two species was placed with a male of the same species as one of the females. The "trio" of flies (two females and one male) was kept together in a 44cc (32mm × 98mm) vial with Wheeler-Clayton medium until one of the females was inseminated. In certain combinations, copulation could be determined by direct observation within an hour or two after the male was placed in the vial with the two females. The actual transfer of sperm was further confirmed by examination of the reproductive tract (vagina and uterus) and the spermathecae and seminal receptacle under a light microscope (100 × magnification). In other combinations where courtship activity or female acceptance response was lower, the trio was left together for a period of six to ten days. After such time, both females per vial were dissected and examined for the presence of sperm.

The data were analyzed by calculation of the Charles-Stalker Isolation Index (Stalker, 1942) which is the ratio of the difference between the frequencies of homogamic and heterogamic matings to the sum of the two types of mating. The index is +1 if sexual isolation is complete, 0 if there is no isolation, and negative if heterogamic matings outnumber the homogamic ones. In the study by Ahearn et al. (1974), the total number of homogamic and heterogamic matings were used in the calculations of the isolation index regardless of whether one, both, or none of the females per vial were inseminated. In this study, for reasons discussed below, the

analysis for sexual isolation between species included trios which had only one of the females inseminated. The number of vials which had both females inseminated or both females virgins was recorded but were not included in the calculation of the isolation indices.

The rationale behind the use of vials which had only one of the females inseminated in the calculation of the isolation index is that in vials which have both females inseminated, the second female to be inseminated may have been the result of a "no-choice" situation. Since females which have been inseminated become aggressively non-receptive to the courtship overtures of the male, the male's overall interaction with the yet-virgin female may be increased. Trios in which both females are found to be virgins after a reasonable period with the male were also not used in the calculation of the isolation index since it is not known whether the male was responsible for non-mating in these vials.

In most cases, since the proportion of homogamic matings far outnumber heterogamic ones, it was not necessary to test the degree of isolation for statistical significance. However, in those cases where the proportion of heterogamic matings approached the proportion of homogamic matings, a test of significance $(C)$ was calculated by examining the distribution of the sample tested in relation to a normal distribution: $C = (\hat{p} - p)/\sqrt{pq/n}$ where $\hat{p}$, the mean of the sampling distribution, is the unbiased estimate of $p$, the mean (0.5) of a normal distribution; and $pq/n$ is the variance of the sampling distribution. Then $C = (\hat{p} - 0.5)/\sqrt{0.25/n} = 2\sqrt{n} \cdot (\hat{p} - 0.5)$. At the 5% confidence interval, the null hypothesis that the two species are mating at random is accepted if $-1.96 < C < +1.96$.

RESULTS

The results of the experiments are reported in Table 1. The data for combinations involving *heteroneura*, *silvestris*,

TABLE 1. *Mating tests (one male and two females) among four species of the* Drosophila planitibia *subgroup.* H = heteroneura, S = silvestris, D = differens, P = planitibia.

| Male × Female | | $n$ | Vials with no matings | Vials with two matings | Homogamic matings only | Heterogamic matings only | $I^1$ | $C^2$ |
|---|---|---|---|---|---|---|---|---|
| H | H,S | 38 | 6 | 5 | 26 | 1 | 0.92 | 4.81 |
| S | H,S | 40 | 12 | 3 | 24 | 1 | 0.92 | 4.60 |
| H | H,P | 43 | 8 | 3 | 32 | 1 | 0.94 | 5.40 |
| P | H,P | 99 | 37 | 27 | 23 | 12 | 0.31 | 1.86 |
| H | H,D | 49 | 11 | 5 | 33 | 0 | 1.00 | 5.74 |
| D | H,D | 103 | 29 | 25 | 24 | 25 | −0.02 | −0.14 |
| S | S,P | 81 | 41 | 3 | 32 | 5 | 0.73 | 4.44 |
| P | S,P | 63 | 21 | 10 | 20 | 12 | 0.25 | 1.41 |
| S | S,D | 42 | 15 | 1 | 25 | 1 | 0.92 | 4.69 |
| D | S,D | 59 | 14 | 10 | 30 | 5 | 0.72 | 4.26 |
| P | P,D | 66 | 5 | 29 | 27 | 5 | 0.68 | 3.85 |
| D | P,D | 103 | 36 | 36 | 12 | 19 | −0.23 | −1.26 |

[1] $I$ = Charles-Stalker Isolation Index.
[2] The null hypothesis that mating is random is accepted when $-1.96 < C < +1.96$.

and *planitibia* were partially extracted from the raw data collected by Ahearn et al. (1974). However, since only trios which had only one female inseminated were used in the calculations of the isolation indices, additional replicates of the experiments reported by Ahearn et al. (1974) were conducted in order to increase sample size.

As reported by Ahearn et al. (1974), the sympatric species, *heteroneura* and *silvestris*, show a pronounced ethological isolation. However, when the Charles-Stalker Isolation Indices ($I$) are re-calculated on the basis of the criteria discussed above, both reciprocals show a value of 0.92 which indicates a much stronger isolation than reported by Ahearn et al. (1974). They reported indices of 0.68 and 0.70 in the two reciprocals. In the experiments involving *heteroneura* and *planitibia*, there is strong ethological isolation in one direction (when *heteroneura* is the male, $I = 0.94$) and a lower isolation in the reciprocal ($I = 0.31$). Between *silvestris* and *planitibia*, the results are similar, strong isolation is observed when *silvestris* is the male (although slightly lower than when *heteroneura* is the male, $I$ in this case is 0.73) and again a much lower isolation in the reciprocal ($I = 0.25$). These data do not seriously conflict with those presented by Ahearn et al. (1974).

The experiments involving *differens*,

the fourth species in this group, show some interesting results. Males of *differens* are readily accepted by females of both *planitibia* ($I = -0.23$) and *heteroneura* ($I = -0.02$). D. *silvestris* females, on the other hand, show relatively strong discrimination against *differens* males ($I = 0.72$). Reciprocally, females of *differens* exhibit strong discrimination against males of *heteroneura* ($I = 1.0$), *silvestris* ($I = 0.92$) and to a lesser extent *planitibia* ($I = 0.68$).

## Discussion

As discussed by Ahearn et al. (1974), two kinds of reproductive isolation mechanisms are operating among the four *planitibia* complex species studied in this paper. Craddock (1974) showed that partial sterility in $F_1$ hybrids (hybrid females are fertile in backcrosses) is the principal isolating barrier between *planitibia* and the other three species. However, she hypothesized that ethological isolation must play a key role in the reproductive isolation between the sympatric species, *heteroneura* and *silvestris*, since both sexes of $F_1$ hybrids and subsequent hybrid generations show no apparent breakdown in viability or fertility from pair matings of *silvestris* female × *heteroneura* male. For the reciprocal cross, she was not able

to obtain successful matings in 36 pairs, a fact which indicates strong sexual isolation between *heteroneura* females and *silvestris* males. Nevertheless, Ahearn and Val (1975) were able to overcome this ethological barrier in the laboratory by placing two virgin *heteroneura* females with one *silvestris* male and were therefore able to study the fertility of this reciprocal combination. They observed that $F_1$ hybrid males are fully fertile. Studies of $F_2$ breakdown, if any, are in progress.

It is a generally accepted hypothesis that premating (e.g. ethological) isolation may be expected to be operating more often between sympatric than between allopatric species as an *ad hoc* product of natural selection while postmating barriers (e.g., hybrid sterility or inviability) may arise as incidental by-products of genetic divergence between incipient species (Dobzhansky, 1970). The results of the ethological data presented in this study can be summarized as in Figure 1. Clearly, females of the sympatric species pair, *heteroneura* and *silvestris*, show strong discrimination in both reciprocal combinations. In allopatric pair comparisons, there is random mating in at least one reciprocal direction except between *differens* and *silvestris* where there is relatively strong sexual isolation in both directions.

For reasons of parsimony and the geological newness of the Island of Hawaii, one can assume that the two Big Island species are the more derived species while *planitibia* and *differens* are the more ancestral. If this assumption is accepted, it is possible to interpret Figure 1 in the following manner. Females of derived species mate randomly with males of ancestral species. However, the converse situation does not hold. Females of ancestral species show strong sexual discrimination against males of the more derived species. For example, *heteroneura* females (derived species) will accept males of both *planitibia* and *differens* but females of *planitibia* and *differens* (ancestral species) strongly discriminate against



--→ = strong isolation
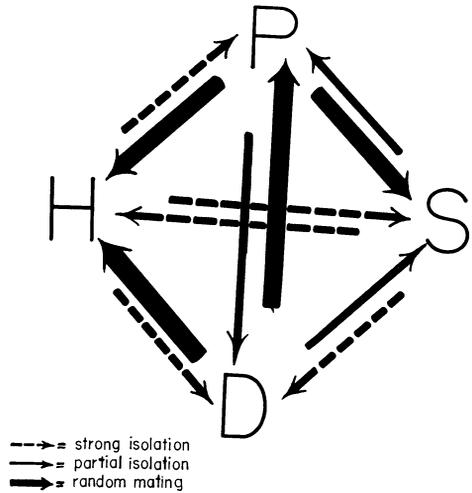—→ = partial isolation
━▶ = random mating

FIG. 1. Pair-wise comparison of sexual isolation between species of the *planitibia* subgroup: P = *planitibia* (Maui), D = *differens* (Molokai), H = *heteroneura* (Hawaii), S = *silvestris* (Hawaii). Arrows point to females and indicate the degree of discrimination by the female.

*heteroneura* males. If this argument is accepted, then it is possible to postulate that *differens* is ancestral to *planitibia*. *D. differens* males are readily accepted by *planitibia* females but not vice versa. This kind of one-sided mating preference between species has not been reported in any other species group.

The founder principle is hypothesized to play a major role in the interisland speciation of Hawaiian *Drosophila* (Carson 1968, 1971). Speculatively, the mechanics of this evolutionary process may provide a basis for justification of the above arguments. A founder individual (single fertilized female) represents only a portion of the total gene pool of the ancestral population. The courtship pattern of the derived species therefore has elements in common with its ancestral population; but on the other hand, a few elements of the total courtship pattern of the ancestral population are changed ("lost") by the "genetic revolution" which accompanies the founder event in the derived population. In this way, females of derived species may recognize
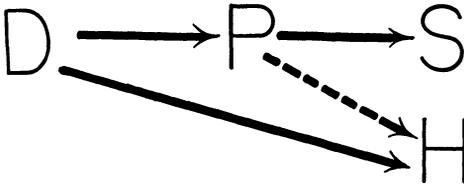
FIG. 2. Postulated phylogenetic relationship of *differens* (D), *planitibia* (P), *silvestris* (S), and *heteroneura* (H) (see text for explanation of dotted arrow).

and accept the courtship overtures of males of ancestral species since these males contain all the courtship elements present in conspecific males. However, females of ancestral species show strong discrimination against males of derived species since these males contain only a portion of the total courtship pattern of conspecific males.

Based on the above arguments, it is possible to formulate a phylogenetic scheme as shown in Figure 2. *D. planitibia* can be derived from *differens*, and *silvestris* from *planitibia*. It is possible to derive *heteroneura* from either *differens* or *planitibia* except that the isolation index between *heteroneura* and *differens* is −0.02 while between *heteroneura* and *planitibia* it is 0.31. It is hoped that future studies on the genetic relationships of all four of these species will supplement and clarify the concepts presented here.

## SUMMARY

Ethological isolation among four species of Hawaiian *Drosophila*, *differens* (Molokai), *planitibia* (Maui), *heteroneura* (Hawaii), and *silvestris* (Hawaii), is measured in "male-choice" experiments. Pronounced sexual isolation is observed between the sympatric species, *heteroneura* and *silvestris*, and further documents the concept that premating isolating mechanisms evolve as *ad hoc* products of natural selection between sympatric species. However, in allopatric combinations, there is a one-sided mating preference such that females of ancestral species are more dis-

criminating against males of derived species than conversely. These observations provide further basis for the interpretation of phylogenetic relationships among these four species and indicate that *differens* is the most probable ancestor of the *planitibia* complex.

### LITERATURE CITED

AHEARN, J. N., H. L. CARSON, TH. DOBZHANSKY, AND K. Y. KANESHIRO. 1974. Ethological isolation among three species of the *planitibia* subgroup of Hawaiian *Drosophila*. Proc. Nat. Acad. Sci. 71:901–903.

AHEARN, J. N., AND F. C. VAL. 1975. Fertile interspecific hybrids of two sympatric Hawaiian *Drosophila*. Genetics 80:s9.

CRADDOCK, E. M. 1974. Reproductive relationships between homosequential species of Hawaiian *Drosophila*. Evolution 28:593–606.

CARSON, H. L. 1968. The population flush and its genetic consequences. *In* R. C. Lewontin (ed.) Population Biology and Evolution. Syracuse Univ. Press, Syracuse, New York. pp. 123–137.

———. 1971. Speciation and the founder principle. Stadler Genetics Symposia 3:51–70.

CARSON, H. L., AND H. D. STALKER. 1968. Polytene chromosome relationships in Hawaiian species of *Drosophila*. II. The *D. planitibia* subgroup. Univ. Texas Publ. 6818:355–366.

DOBZHANSKY, TH. 1970. Genetics of the Evolutionary Process. Columbia Univ. Press, New York. 505 pp.

JOHNSON, W. E., H. L. CARSON, K. Y. KANESHIRO, W. W. M. STEINER, AND M. M. COOPER. 1975. Genetic variation in Hawaiian *Drosophila* II. Allozymic differentiation in the *D. planitibia* subgroup. *In* Isozymes IV Genetics and Evolution. C. L. Markert (ed.) Academic Press Inc., New York. pp. 563–584.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Publ. 7213:145–153.

SPIETH, H. T. 1966. Courtship behavior of Hawaiian Drosophilidae. Univ. Texas Publ. 6615: 245–313.

——. 1968a. Evolutionary implications of sexual behavior in *Drosophila*. *In* Th. Dobzhansky, M. K. Hecht, and W. C. Steere (eds.), Evolutionary Biology. Appleton-Century-Crofts, New York, pp. 157–193.

——. 1968b. Evolutionary implications of the mating behavior of the species of *Antopocerus* (Drosophilidae) in Hawaii. Univ. Texas Publ. 6818:319–333.

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

WHEELER, M. R., AND F. E. CLAYTON. 1965. A new *Drosophila* culture technique. Dros. Info. Serv. 40:98.

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