NATURAL HYBRIDIZATION BETWEEN THE SYMPATRIC HAWAIIAN SPECIES *DROSOPHILA SILVESTRIS* AND *DROSOPHILA HETERONEURA*

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Abstract.—Two newly formed, morphologically distinct species of *Drosophila* from the island of Hawaii have been found to form fertile hybrids in two areas of sympatry. Both F_1 and backcross hybrids have been recognized in nature; in one case, the hybridization events extended over three years. Original hybridizations involved one or more *D. silvestris* females mating with *D. heteroneura* males. Female F_1 hybrids from this cross have participated in backcrosses to *D. silvestris*. In any one locality, less than 2% hybrids have been found in nature. A hybrid swarm was not formed; selection appears to favor a strict maintenence of morphologies characteristic of the separate species. This result is attributed to pervasive sexual selection, which serves to preserve the syndromes of sexual characteristics that arose during past allopatric divergence. Populations of *D. silvestris* both within and outside the present range of *D. heteroneura* often display heritable variation in color patterns involving the abdomen, pleurae, legs, and wings. Genes effecting variation in these characters may be derived from genes involved in a past introgression from *D. heteroneura*. Independent evidence for past hybridization between these species comes from study of mitochondrial DNA. Although the inferred direction of the cross is the opposite of that observed in the recent case described here, both reciprocal crosses have been obtained experimentally in the laboratory. Accordingly, we suggest that these species may have been open to hybridization since their first sympatic encounters following their inception in allopatry. That they remain as strictly recognizable morphological entities is due both to their current partial allopatry and to the action of sexual selection in maintaining two separate major modes of efficient reproduction. There is no reason to invoke specific reinforcing selection that has imposed reproductive isolation.

Hybridization in nature between fully differentiated, morphologically distinct species is not uncommon. Nevertheless, the overall genetic integrity of the participating species is rarely threatened, although it may be altered locally. It is widely theorized (e.g., Mayr, 1963) that the species is best defined as an inviolate genetic unit that is maintained by the alleged tightness of various mechanisms that isolate it reproductively. These are supposed to have been brought about by natural selection that favors genetic integrity.

Although the above view has classical status in evolutionary biology, there are surprisingly few data that compel its adoption. An alternate possibility is simply that the gene pool of each species is held separate from others primarily by the positive force of a strong balancing selection that operates within each species to maintain its own optimal reproductive mode (Paterson, 1978, 1981). Sexual selection, a wholly intraspecific reproductive mode that is not concerned with isolation, is an example of such a positive selective force (Carson, 1986a). A temporary loosening of this mode may nevertheless allow hybrids to be formed occasionally in a local population. Following hybridization, disturbance to the gene pool is usually quite limited geographically, and renewed selection may soon reestablish the optimal reproductive mode.

Under the above circumstances, even substantial amounts of hybridization in some areas of sympatry will not destroy the genetic architecture of either participating species. In such a scenario, the genetic in-

tegrity of the species is maintained without recourse to selection that favors some sort of "isolating mechanism." Indeed, hybridization may serve a positive role, since it can lead to introgression of foreign genes into a species, thus adding to the genetic variability of the gene pool of the recipient. Opportunities for novel recombination followed by selection may thus arise (Anderson, 1949). According to this view, the appearance of isolation may be judged to be an incidental effect.

The cases of hybridization described here supplement previous brief accounts (Kaneshiro and Val, 1977; Carson et al., 1986). They involve a pair of relatively new, partially sympatric Hawaiian drosophilids, *Drosophila silvestris* and *Drosophila heteroneura*. The species differ principally in morphological attributes that reflect their differing modes of sexual selection rather than differential adaptation to the ambient environment. Differences arising through sexual selection have been generally inferred to exist within many Hawaiian *Drosophila* species (Ringo, 1977; Carson, 1978). Sexual selection has recently been confirmed experimentally for *D. silvestris* in particular (Carson and Teramoto, 1980; Spiess and Carson, 1981; Carson, 1986a). We show here that fertile F_1 and backcross hybrids between the two species are formed in nature. Despite the occurrence of 1.7% hybrids in one area, sexual selection has apparently continued to favor each of the two strongly differing phenotypic morphological modes of the two species, effectively maintaining the distinctness of the participating species.

MATERIALS AND METHODS

Drosophila silvestris and *D. heteroneura* are endemic to the "Big Island" of Hawaii, where they have been found sympatrically at seven sites, mostly at altitudes between 1,000 and 1,200 m on both the Hilo and Kona sides of this island (Fig. 1). The two species are morphologically distinct, especially the males (Fig. 2; see also Hardy [1965], Val [1977], and Carson [1978] for detailed illustrations). On the Hilo side of the diagonal line in Figure 1, male specimens of *D. silvestris* display a qualitative difference from conspecifics on the opposite

ISLAND OF HAWAII

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0        20      40
      KILOMETERS
   Contours in Meters
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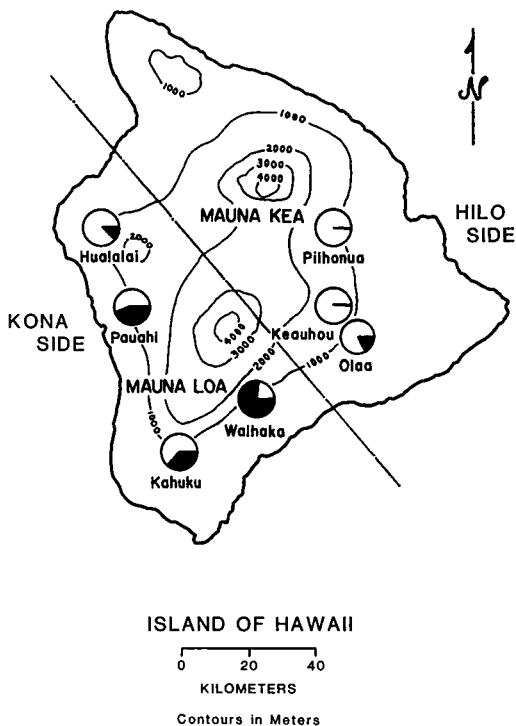
FIG. 1. Map of the island of Hawaii, showing seven sites where *Drosophila silvestris* and *D. heteroneura* have been found in sympatric association. Each site is represented by a circle; the black portion represents the proportion of *D. heteroneura* found in the population. The diagonal line separates the more ancestral Kona-side populations of *D. silvestris* from the derived Hilo-side populations (see text).

(Kona) side. This difference involves an apomorphic secondary sexual character. Hilo-side males have 25–30 additional long cilia on the dorsum of each foretibia, compared with Kona-side specimens. The legs of *D. heteroneura* resemble, but are not identical to, those of the Kona populations of *D. silvestris* and are not geographically differentiated (Carson et al., 1982).

Both species tend to be found in the vicinity of their principal host plants, tree-form lobeliads of the genus *Clermontia*. Both, however, will also oviposit and complete the life cycle on decaying bark of the common rainforest tree *Cheirodendron gaudichaudii*. Being large and easy to see, both species of flies can be collected without baits as they rest at lek or oviposition sites. Nevertheless, most of the specimens on which this study is based were caught at artificially prepared baits consisting of fer-
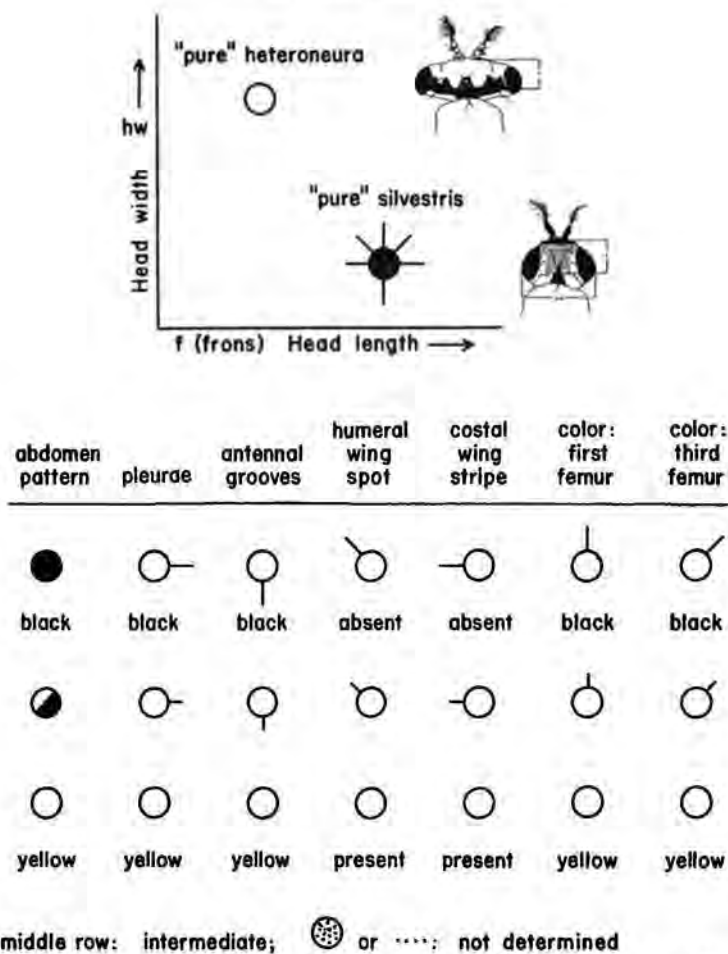
FIG. 2. Diagrams explaining the construction of the pictorialized scatter diagrams employed to represent the phenotypes of individual specimens in subsequent figures. An exemplary basic plot of two male individuals is shown at the top of the figure, with head width (hw; relatively large in *D. heteroneura*) plotted against a frons measurement (f; head length). The rest of the characters are diagrammed as shown in the lower part of the figure. The glyphs can be used to represent either sex, with the exception of the color of the antennal grooves (vertical spike at the bottom of the symbol) for which a difference exists only between males. The females have yellow or dull yellow antennal grooves in both species.

mented bananas or commercial mush-rooms. In the areas of sympatry, adult members of these two species frequently en-counter one another in the microenviron-ment; sometimes the leks of the males of the two species occur on opposite sides of the same small tree (Conant, 1978). Fe-males of the two species are sometimes found together at an oviposition site; ima-goes of both species have been reared from naturally oviposited eggs in the same small (50 cm long) branch of the host plant, in-dicating little differentiation between the species in oviposition site. At altitudes above

1,400 m, however, and extending up to about 1,600 m, *D. silvestris* occurs alone. Populations consisting of *D. heteroneura* only have not been found.

In the present study, each specimen col-lected at sympatric sites in the wild was ex-amined for pigmentation and morphology. Morphological inspection is the most sat-isfactory way of distinguishing the species; neither has fixed (diagnostic) electropho-retic alleles or inversions (Sene and Carson, 1977; Craddock and Johnson, 1979). Each species tends to have a distinctive syndrome of characters, making identification of

TABLE 1. Frequencies of wild-caught specimens of *D. silvestris* and *D. heteroneura* in areas where the species are sympatric (1970–1986).

Locality	D. silvestris		D. heteroneura		Total	Percentage D. hetero- neura	Hybrids	
	Males	Females	Males	Females			Number	Percentage
Kona side:								
Pauahi	235	193	180	167	775	44.8	0	0
Hualalai	284	165	56	9	514	12.6	0	0
Kahuku Ranch	300	361	250	135	1,046	36.8	18	1.7
Waihaka	19	11	71	31	132	77.3	0	0
Hilo side:								
Olaa	444	517	114	91	1,166	17.6	1	0.09
Keauhou Ranch	213	310	3	5	531	1.5	0	0
Piihonua	151	112	6	1	270	2.6	0	0
Total:					4,434	25.2		

species unequivocal. Ease of morphological recognition of hybrids among wild-caught specimens was enhanced by the work of Val (1977), who had obtained abundant fertile hybrids (F_1, F_2, and backcross) from reciprocal crosses between these species in the laboratory.

The wild-caught specimens screened in this study are listed in Table 1. All but one of the naturally occurring hybrids was found at moderate altitudes in the Kahuku Ranch area (Table 2). Hybrid specimens in the latter area were found over a three-year period from 1974 to 1977. Among 227 specimens collected at the same site over the subsequent year and a half, no further hybrids were found. More recently, we have been unable to collect any specimens of either species in the area. Difficulty in collecting these species, especially at the lower altitudes, has been widely encountered on the island of Hawaii since 1982. Environmental decline due to El Niño-induced drought conditions, destruction of the forests by man, and the arrival of a new hymenopteran predator, *Vespula pennsylvanica,* have all been advanced as partial explanation of this population decline. Whatever the cause, the recent decline of these species in collections was not confined to the Kahuku Ranch area.

In addition to the wild specimens listed in Table 2, several progenies of single isolated wild females (inseminated in nature) have been reared in the laboratory, mor-

TABLE 2. Numbers of specimens captured at Kahuku Ranch, Island of Hawaii during 1974–1985. Sites: a = 1,160 m site; b = 1,240 m site (see text).

Collection number	Site	Date	D. silvestris		D. heteroneura		Hybrid	
			Male	Female	Male	Female	Male	Female
T22	a	10/6/74	0	0	0	0	0	0
T32	a	12/26/74	0	0	5	6	1	0
T39	a	2/14/75	4	4	22	36	0	0
T51	a	5/20/75	4	9	6	38	1	0
T68	a	8/12/75	17	7	16	19	1	0
T69	b	8/14/75	28	52	9	36	1	1
T70	b	8/14/75	46	25	22	30	1	*
T71	b	8/14/75	5	6	0	1	0	0
T96	b	4/14/76	143	55	22	13	10	0
U26	b	1/18/77	43	54	11	25	0	2
U41	b	7/19/77	43	47	13	29	0	0
U50	b	2/1/78	20	28	9	14	0	0
U62	b	8/2/78	9	12	0	3	0	0
W22	b	3/22/82	0	0	0	0	0	0
W70	a, b	3/7/85	0	0	0	0	0	0
Totals			362	299	135	250	15	3

* One *silvestris* female was proved by progeny tests to have mated with a *heteroneura* male.

phologically screened, and measured. As will be documented, some of these produced hybrid progenies.

Visualization of some aspects of the morphological and color variability has been carried out by making use of plots of pictorial scattergrams according to the method of Anderson (1949). The mode of notation is explained in Figure 2; the relevant characters are illustrated in more detail in Val (1977), from which the pictorial insets of the male heads in Figure 2 have been taken. As in Val (1977), measurements of the heads were made with a Bausch and Lomb micrometer disc (100 divisions) placed in the right-hand $10 \times$ wide-field ocular of a Bausch and Lomb stereomicroscope with the pod magnifier zoomed to $3 \times$, providing a magnification of $30 \times$. Under these circumstances, one micrometer unit equals 0.03303 mm.

RESULTS

The basic data of this study are presented in Table 3; some of these sets are further illustrated by scattergrams in Figures 3–6. Mutual interference in the figures of individuals with similar head shape has been avoided by entering some of the data points in the form of smaller closed or open symbols representing only head shape and abdomen color. Table 3 records numerical and statistical data on the head measurements in micrometer units, as defined above.

In order to provide a broad perspective on the findings in the Kahuku Ranch area, scattergrams representing specimens from various other Kona-side and Hilo-side populations are also presented. Characters other than the head measurements (Fig. 2) cannot be dealt with in a quantitative manner; nevertheless, variability occurs within both species and especially in *D. silvestris*. For example, Figure 3A, B, and C shows variability in costal wing stripe within both species at both Pauahi and Hualalai; *D. silvestris* also shows variable abdomen and foreleg color. In these populations, however, neither species shows the head shape, antennal groove (face) color, or pleural color characteristic of laboratory-produced F_1 hybrid males (see Val, 1977). Head width and head length were strongly correlated (little scatter about regression line) in both

males and females in these populations. Similar facts are revealed by the scattergrams of the preponderance of wild specimens caught at the Kahuku Ranch (Fig. 3D, E).

Most important in this regard are 14 wild males from the Kahuku collections that are diagrammed in Figure 3F. On this figure, the regression lines for head measurements for putatively nonhybrid males are given, reproduced from Figure 3D. All but two of these males show the characteristic face (antennal groove) color of laboratory-obtained F_1 hybrid males. This includes the six males closest to the *D. silvestris* regression line; were it not for the face color, three of these might not have otherwise been recognized as hybrids. From earlier work on laboratory hybrids (Val, 1977), there is every reason to expect all of these males to be fertile; in fact, two of them were tested and proved to be fertile when they were crossed separately to laboratory virgin females of first one species and then the other.

As routine procedure, the sexes of wild-caught flies were separated at capture, and most of the wild females appearing to be either *D. silvestris* or *D. heteroneura* were placed into separate culture vials upon reaching the laboratory. Thus, we were able to rear isofemale progeny representing natural inseminations. We assayed 144 of these progenies for chromosomal variability; there are four polymorphic chromosome inversions segregating in the population (Carson and Bryant, 1979).

Most of these isofemale progenies were discarded at the larval stage, but in 13 instances, F_1 imagoes were reared. As a baseline for the study of morphological variability, we present measurements of several apparently unhybridized isofemale progenies (Fig. 4A, B); these may thus serve as controls. In two cases in which the mother had been identified as *D. silvestris,* many of the progeny nevertheless deviated from the norms of the two species (compare Fig. 4C–F with Fig. 4A, B). To facilitate comparison with the controls, the regression lines from Figure 4A and B are reproduced in Figure 4C–F.

The progeny illustrated in Figure 4C and F (isofemale T70B13) appears to represent a mating of a *D. silvestris* female with a *D. heteroneura* male. The narrow distribution

TABLE 3. Correlation analysis of head measurements of *D. heteroneura, D. silvestris,* and their hybrids. Generation: 0 = wild-caught; 1 = first laboratory generation. For collection numbers not listed in Table 2, the year of collection is given in parentheses. Collection numbers given for first-laboratory-generation samples consist of the collection number of the wild-caught flies followed by an isofemale-line number. Measurements are in micrometer units; for conversion to millimeters, see Material and Methods. b = coefficient of regression of head width on head length; SE = standard error of regression coefficient; r = correlation coefficient.

| Locality	Genera-tion	Collection number	N	Head length		Head width		Regression ± SE		
				Mean	Vari-ance	Mean	Vari-ance	b	SE	r
D. heteroneura males:										
Pauahi	0	S39 (1973)	20	22.43	2.81	88.10	70.79	4.62	1.18	0.92
Hualalai	0	U5 (1976)	16	20.75	1.63	82.84	18.84	2.21	0.91	0.65
Kahuku	0	T32, T39	27	23.11	3.32	84.80	85.24	4.58	1.01	0.90
Kahuku	0	T68, T69, T70	30	22.27	5.13	82.42	89.31	3.88	0.79	0.93
Kahuku	0	T96	19	23.00	1.26	87.16	31.37	3.60	1.21	0.72
Kahuku	0	U26	9	21.44	2.06	81.72	52.43	4.60	1.91	0.91
Olaa	0	U13 (1976)	20	20.51	2.73	81.83	35.82	2.50	0.85	0.69
Pauahi	1	S39H7	17	21.91	2.96	83.85	50.39	3.83	1.07	0.93
Kahuku	1	U41N4	27	23.56	1.53	85.37	11.30	2.25	0.54	0.82
D. heteroneura females:										
Pauahi	0	S39 (1973)	20	20.55	0.55	62.90	9.85	3.13	1.00	0.74
Kahuku	0	T32, T39	31	20.81	2.82	61.44	31.62	3.02	0.62	0.90
Kahuku	0	T69	8	19.75	2.62	59.94	19.14	2.49	1.10	0.92
Kahuku	0	T96	10	19.70	3.34	58.05	39.17	3.26	0.18	0.95
Pauahi	1	S39H7	15	20.90	1.04	63.23	11.46	2.56	0.93	0.79
Kahuku	1	U41N4	27	20.56	0.92	62.78	10.21	3.06	0.67	0.92
D. silvestris males:										
Pauahi	0	S39 (1973)	20	27.23	4.81	57.28	16.44	1.80	0.44	0.97
Hualalai	0	U5	20	27.28	7.66	57.75	22.53	1.65	0.40	0.96
Kahuku	0	T96	20	28.30	11.64	58.23	42.32	1.87	0.45	0.98
Kahuku	0	U26	38	28.11	4.62	58.28	14.10	1.53	0.29	0.87
Kahuku	0	U50	20	27.73	5.69	56.95	13.21	1.45	0.36	0.95
Keauhou	0	S73 (1974)	20	29.20	7.85	61.58	27.74	1.81	0.44	0.96
Kilauea	0	S43 (1973)	20	29.03	8.26	60.25	21.59	1.55	0.38	0.96
Pauahi	1	S39H16 (1973)	19	25.58	6.34	54.97	24.96	1.92	0.48	0.97
Kahuku	1	U41N32	23	25.33	1.01	54.41	2.10	1.13	0.31	0.79
Keauhou	1	T93V27 (1976)	20	24.80	1.86	53.78	6.53	1.64	0.44	0.87
Kilauea	1	T92G2 (1976)	23	23.63	1.31	52.30	5.31	1.74	0.44	0.87
D. silvestris females:										
Pauahi	0	S39 (1973)	20	23.78	5.05	53.50	24.65	2.16	0.52	0.98
Kahuku	0	T96	29	25.36	3.88	54.84	13.16	1.79	0.36	0.96
Keauhou	0	S73 (1974)	9	25.33	4.73	55.39	16.63	1.85	0.71	0.99
Kilauea	0	S43 (1973)	20	24.80	5.31	55.00	21.72	1.95	0.48	0.96
Pauahi	1	S39H16	18	21.78	6.27	48.75	21.19	1.76	0.46	0.96
Kahuku	1	U41N32	25	23.00	0.68	50.90	3.56	1.92	0.48	0.83
Keauhou	1	T93V27	20	21.98	2.09	50.00	8.95	1.80	0.48	0.87
Kilauea	1	T92G2	32	21.53	1.00	49.55	3.75	1.71	0.35	0.88
Hybrid males:										
Kahuku	0	(1974–1976)	14	24.93	6.42	59.89	28.13	0.13	0.60	0.06
Kahuku	1	T69B4	14	25.57	3.51	54.75	6.00	1.21	0.38	0.93
Kahuku	1	T70B13	20	24.10	0.79	60.10	1.48	1.14	0.32	0.83
Kahuku	1	U26B1	11	24.09	3.40	60.86	22.22	1.38	0.85	0.54
Kahuku	1	U26B2	32	23.94	6.39	58.66	21.55	1.43	0.34	0.78
Hybrid females:										
Kahuku	1	T69B4	16	20.69	3.23	49.13	6.10	0.81	0.37	0.59
Kahuku	1	T70B13	12	21.33	1.45	57.25	7.56	−0.07	0.72	−0.03
Kahuku	1	U26B1	12	20.92	2.87	53.00	2.63	0.33	0.30	0.34
Kahuku	1	U26B2	28	20.54	3.49	52.46	5.34	0.58	0.24	0.47
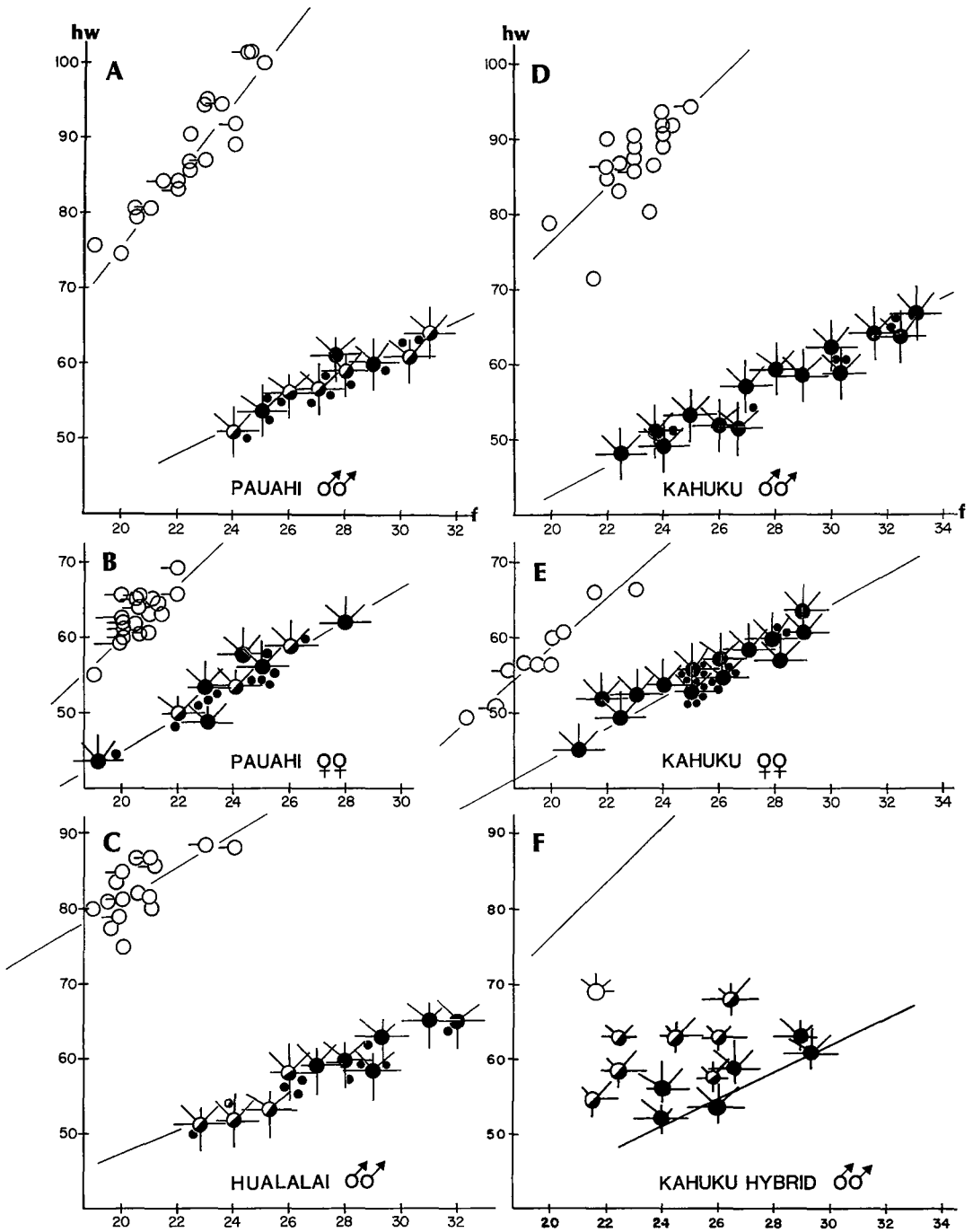
FIG. 3.   Morphology of randomly chosen wild specimens. In all graphs, the ordinate indicates head width, and the abscissa indicates head length. Measurements are in micrometer units; for conversion to millimeters, see text. A) Pauahi males; B) Pauahi females; C) Hualalai males; D) Kahuku males (collection T96 in Tables 2 and 3); E) Kahuku females (collection T96 in Tables 2 and 3); F) 14 selected wild male hybrids from the Kahuku collections, including apparently both F_1 and backcross individuals (see text). For explanation of symbols, see Figure 2. In some cases in which two glyphs overlap, the head measurements and abdomen color only are represented by a smaller dot.
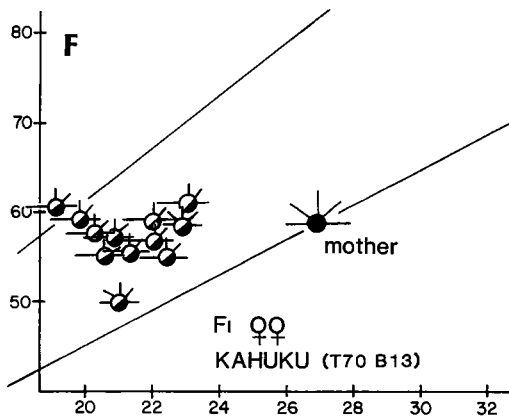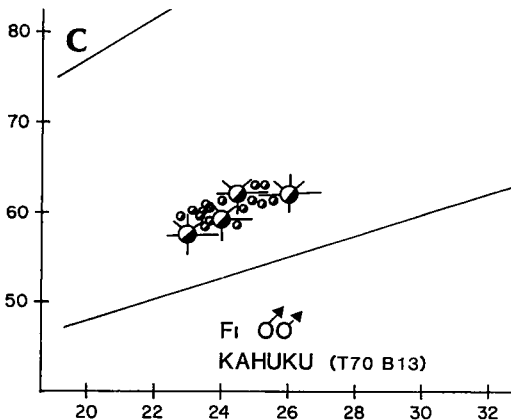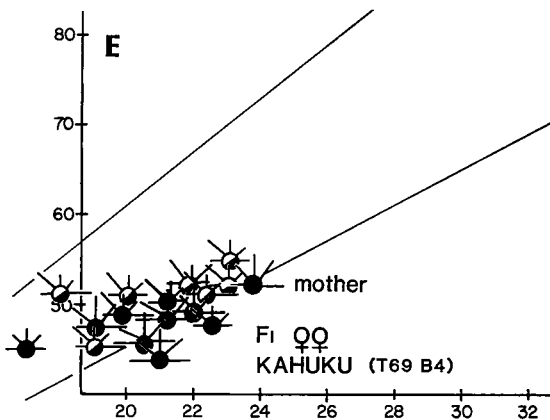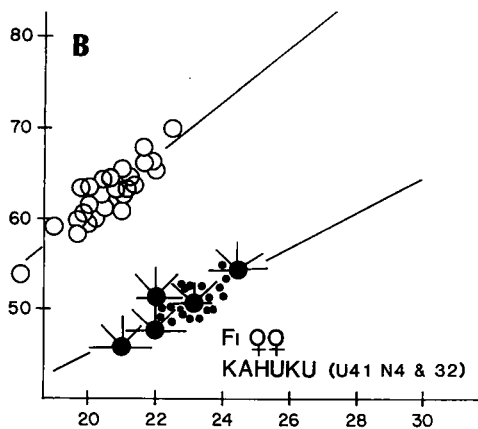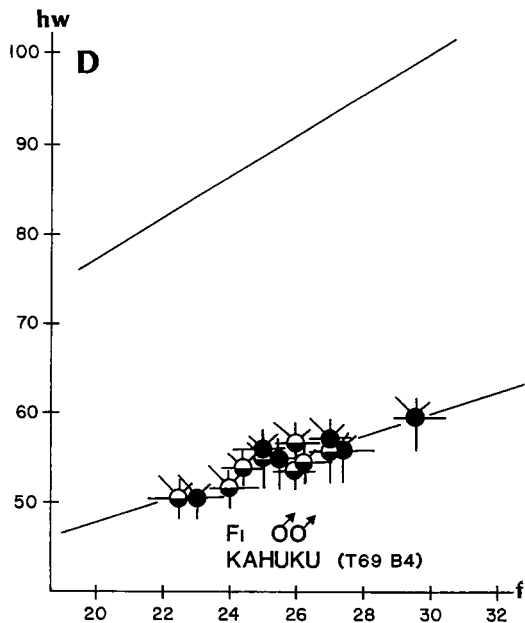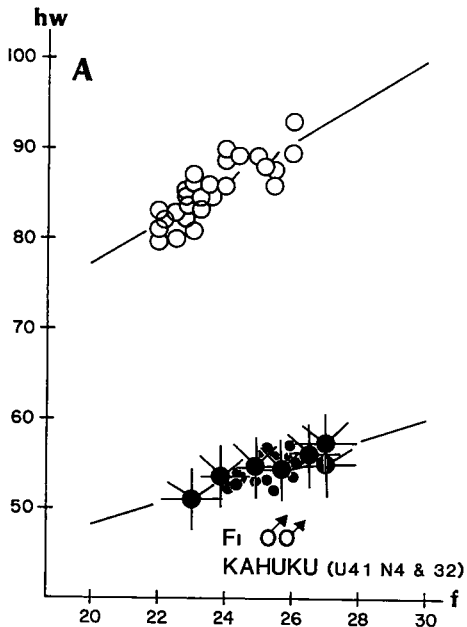
FIG. 4. F₁ progenies from isofemale lines derived from wild females captured at Kahuku Ranch. In all graphs, the ordinate indicates head width, and the abscissa indicates head length; measurements are in micrometer units; for conversion to millimeters, see text. A, B) control progenies showing no evidence of hybridization; C, F) progeny of isofemale T70B13; D, E: progeny of isofemale T69B4. In E and F, the phenotype of the mother, which was indistinguishable from *D. silvestris,* is plotted. In C–F, the control (nonhybrid) regression lines are shown.

of head shapes of F_1 males closely resembles the results of the *D. silvestris* ♀ × *D. heteroneura* ♂ laboratory cross performed by Val (see fig. 2 in Val [1977]). The female progeny of this cross show a stronger effect of the *D. heteroneura* head shape; that is, the mean of the female progeny is near the midparent mean (Fig. 4F, Table 3), as was also found in laboratory crosses by Templeton (1977).

Female T69B4, however, produced F_1 individuals that are best interpreted as involving the cross of a *D. silvestris* male with an F_1 "SH" female (a hybrid between a *D. silvestris* female and a *D. heteroneura* male; in such a formula as "SH," the species symbol of the female parent is given first). In this case, the male progeny have head shapes close to those of *D. silvestris,* yet their hybrid nature is revealed by the segregation of black (*D. silvestris*-like) and intermediate face colors. Of the 14 F_1 male specimens observed in the progeny of female T69B4, eight showed the intermediate face color; the others were black. As expected, the F_1 females show head shapes closer to *D. silvestris* than in the case of the progeny of female T70B13. Segregation of abdominal pattern is also observed in the progeny of female T69B4: there were 14 black and 16 intermediate individuals, with no difference in ratio between the sexes.

Two further cases (progeny of females U26B1 and U26B2; Fig. 5) were also instructive. Unlike the other two cases, the two wild females both showed hybrid characteristics but nevertheless resembled unhybridized *D. heteroneura* in head shape. Their phenotypes match the most *D. heteroneura*-like members of SH female hybrids (see Fig. 4F). The phenotypes of their progeny in the laboratory bear out the initial identification of the wild females (Fig. 5A–D). The progeny of both of these crosses appear to conform to the SH ♀ × *D. silvestris* ♂ backcross in Val (1977). After January 18, 1977, no further putative hybrids were found among specimens captured at Kahuku Ranch (Table 2).

As recorded in Tables 1 and 3, specimens from populations on the Hilo side of the island have also been examined. Phenotypes of a sampling of wild and F_1 specimens from these areas are given in Figure 6. The populations show individual varia-

tion in abdomen pattern and wing and leg markings, as did flies from the Kona side of the island. *D. silvestris* also exhibits variation in these characters in areas outside of the present range of *D. heteroneura* (e.g., Kilauea [Fig. 6B, C]); this locality lies at an altitude about 200 m above the Keauhou site (see Carson and Bryant, 1979).

In these populations, *D. silvestris* manifests varying degrees of expression of yellow on the abdomen (not represented in the figures in this paper). Abdomen color varies from a strongly contrasting yellow and black pattern, similar to that of *D. heteroneura,* to very reduced, ill-defined yellowish blotches on a black or brownish background. That this variation is heritable is also suggested by certain observations of one of us (K.Y.K.). Using an isofemale laboratory stock of *D. silvestris* (T94B7) from the Olaa area, he culled individuals with patterned abdomens over several generations; this resulted in a stock in which the abdomen was permanently black without yellow pattern.

Among over 1,900 specimens from the areas of sympatry on the Hilo side of the island, only a single putative hybrid was found; this was a male specimen (see Fig. 6A) collected at Olaa Tract, Hawaii Volcanoes National Park on August 1, 1980, by W. P. Mull. This specimen shows F_1 hybrid characteristics in face color, like the hybrids from the Kona side of the island and unlike the other intraspecific variability mentioned above. Comparing it with laboratory hybrids obtained by Val (1977) and the wild hybrid males from Kahuku Ranch, it does not appear to be a simple F_1 interspecific hybrid. Although the head shape does not differ much from that of hybrid males with a *D. heteroneura* mother, this specimen displays 21 long cilia on the dorsal surface of the foreleg tibia, whereas five or fewer are expected (Bryant and Carson, 1979). Accordingly, this specimen appears more likely to have resulted from the backcross of an SH female to a *D. silvestris* male.

As indicated earlier, our ability to obtain these species in nature, especially at the lower elevations, declined after 1980. For example, although *D. silvestris* is currently present in substantial numbers at elevations higher than Keauhou, neither species has
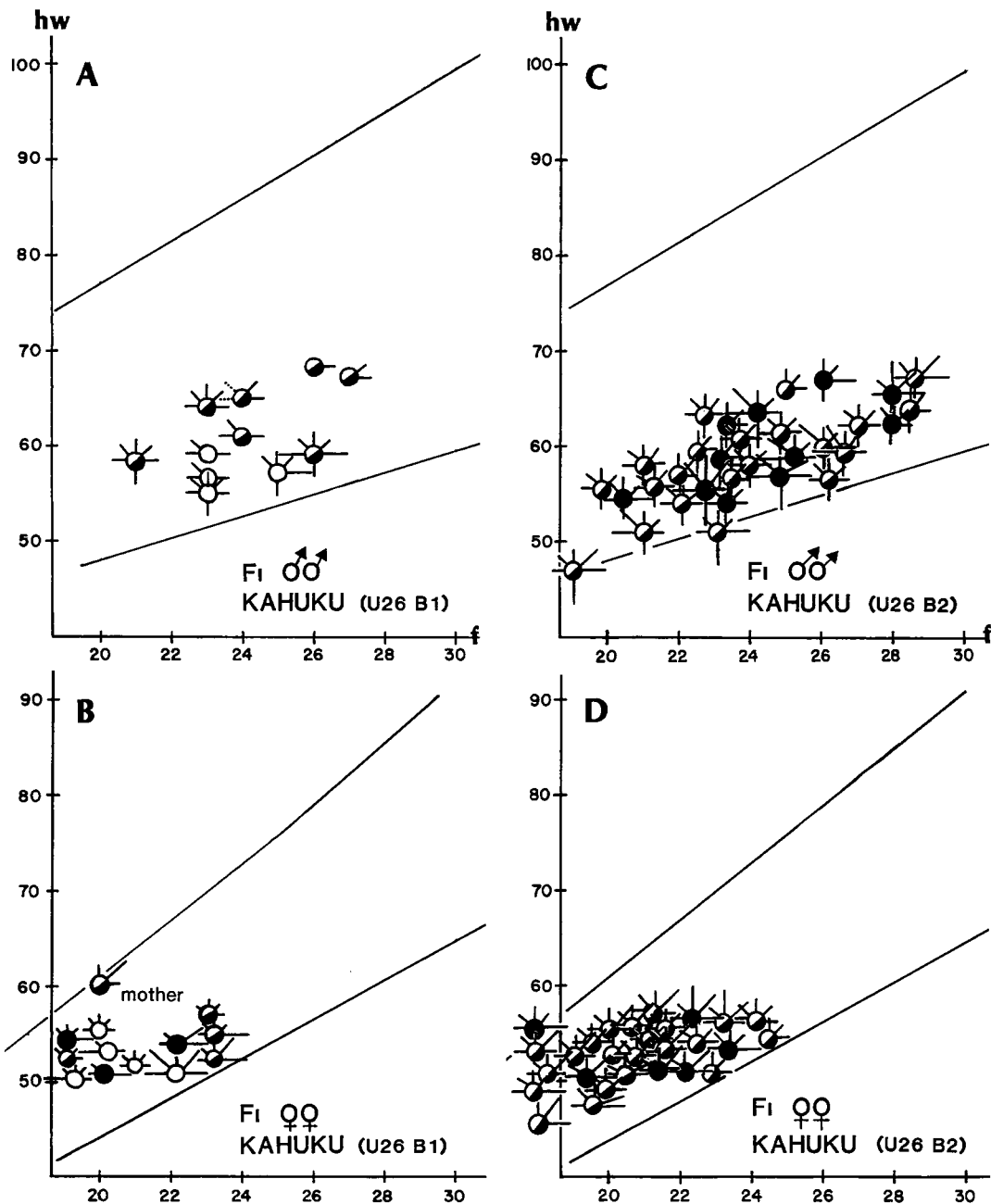
FIG. 5. F_1 progenies from two putative hybrid wild Kahuku Ranch females that were more *D. heteroneura*-like than *D. silvestris*-like. The ordinate indicates head width, and the abscissa indicates head length; A, B) Progeny of isofemale U26B1, whose phenotype is also given; C, D) progeny of isofemale U26B2. The regression lines represent control (nonhybrid) phenotypes.

been collected since 1981 at either Olaa or Keauhou (Fig. 1), and only *D. silvestris* has been collected at Piihonua. The last known specimens of *D. heteroneura* to be collected on the Hilo side of the island were captured on February 15, 1981, in the Olaa Tract behind the Volcano Agricultural Experiment Station.
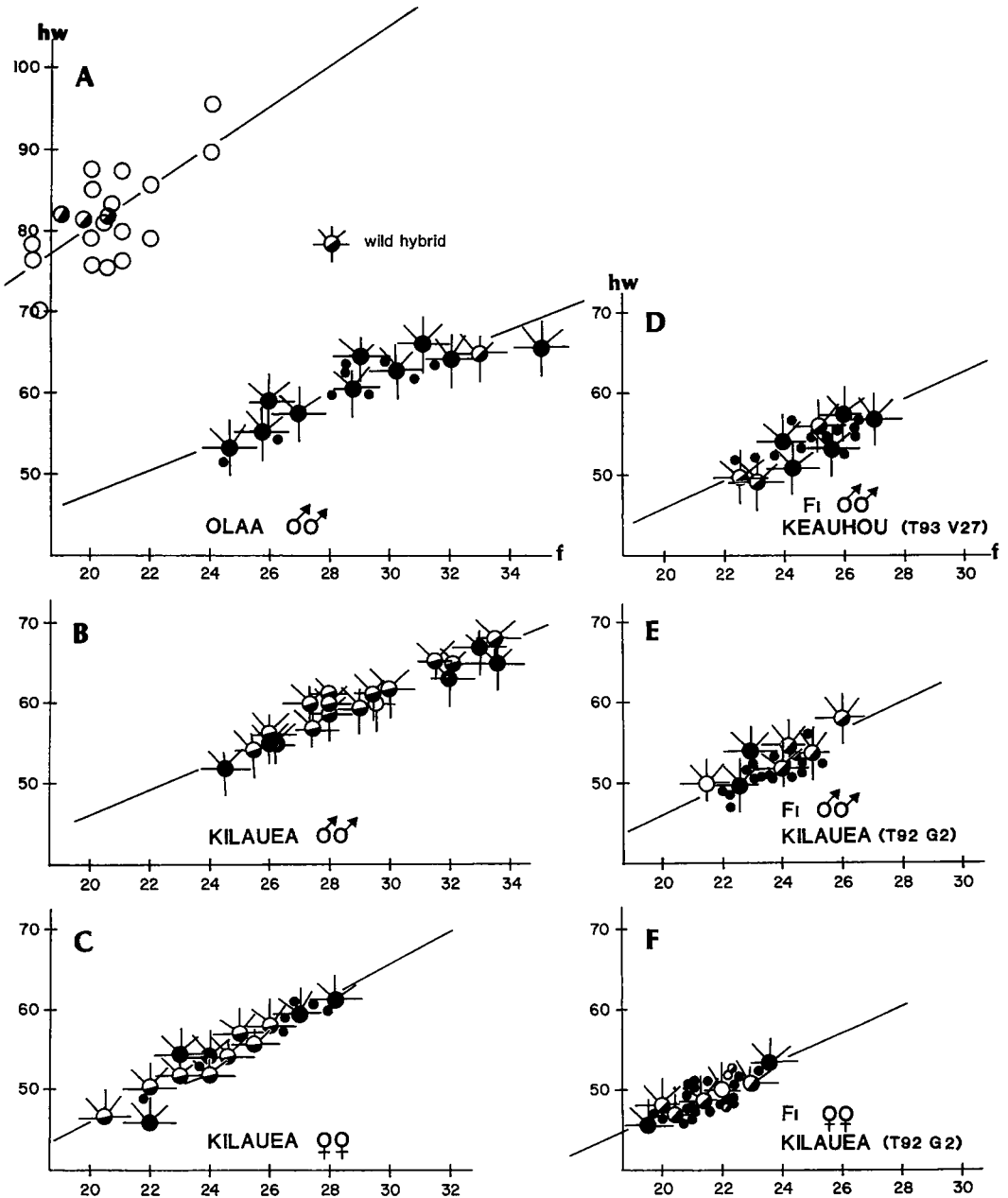
FIG. 6. Hilo-side population samples of *D. silvestris* and *D. heteroneura*. In all graphs, the ordinate indicates head width, and the abscissa indicates head length. A) Wild males of the two species from Olaa, showing the position of a single backcross hybrid male captured in nature; B, C) wild *D. silvestris* from Kilauea Forest Reserve, an area outside the range of *D. heteroneura*; D) F_1 progeny of female T93V27 (*D. silvestris* from Keauhou Ranch); E, F) F_1 progeny of female T92G2 (*D. silvestris* from Kilauea Forest Reserve).

DISCUSSION

The considerable morphological and color differences between *Drosophila silvestris* and *D. heteroneura* are based on a number of gene differences, perhaps as many as 19

(Val, 1977; Templeton, 1977). Although several of these appear to involve genes of major effect, their action is apparently considerably modified by polygenes. The species are so distinct morphologically that the rec-

ognition of naturally occurring hybrids poses no difficulty. The series of reciprocal F_1's, F_2's, and backcrosses performed by Val (1977) provide a firm basis for the interpretation of the details of the natural hybridizations that have occurred at Kahuku Ranch and Olaa Tract.

Hybrids were present in the populations at Kahuku Ranch over a period of three years. Based on the phenotypes of wild-caught hybrid males, the initial hybridization(s) involved *D. silvestris* females mating with *D. heteroneura* males. In one later instance (female T70B13), progeny tests indicated that the same type of cross had occurred. All of the 14 hybrid males recognized among the wild flies collected, with the possible exception of the most *D. heteroneura*-like male (see Fig. 3F), have phenotypes indicating that they are either F_1 SH hybrids or backcross progeny of SH hybrid females and *D. silvestris* males; the single Olaa hybrid appears also to have resulted from this type of backcross.

The backcrosses of hybrid SH females to *D. silvestris* produce some flies, both males and females, that have head shapes and face colors that are not distinguishable from those of unhybridized *D. silvestris* specimens. This suggests that some backcross hybrids might have been missed among the more than 600 flies recorded as being unhybridized *D. silvestris*. For this reason the number of hybrids recorded in Figure 1 should be considered as a minimum. In the relevant backcross (illustrated in Fig. 4D), both face and abdominal color are segregating, apparently independently. Thus, not more than one in four males would be expected to have both the face and abdomen color causing them to be mistaken for *D. silvestris*. The fact that the hybridizations in nature appear to involve *D. silvestris* rather than *D. heteroneura* females is compatible with the observation that *silvestris* females hybridize in the laboratory more easily than *D. heteroneura* females (Val, 1977).

Accordingly, it appears that no hybrid swarm has been formed and that the observed hybridizations result largely in introgression of *D. heteroneura* genes into *D. silvestris*. There is, however, no evidence that face color and head width, the key sexual differences between the species, are involved in the introgression.

In view of full mating ability and fertility of hybrids as established in laboratory tests (Ahearn, 1980; Ahearn and Templeton, 1988), what has prevented the formation of a hybrid swarm? Sexual selection appears to be strongly developed in these species (Carson, 1978, 1986a); this is further borne out by experiments using *D. silvestris* (Spiess and Carson, 1981). For example, only about one-third of the *D. silvestris* males are successful in competition for mates in laboratory experiments (Carson, 1986a). Unsuccessful males nevertheless court vigorously and have abundant motile sperm. Heterokaryotypes exist in disproportionately high frequency among the successful males (Carson, 1987).

We favor the view that the strong sexual-selection systems of the two species effectively keep the two gene pools relatively intact. Thus, even though interspecific laboratory hybrids produce abundant progeny in pair matings, we suggest that in nature the mate-choice system serves to exclude most of them from reproduction.

What conditions are conducive to hybridization? The environment at Kahuku Ranch where the hybridizations took place may have played an important role. The first three male hybrids were captured at an altitude of 1,160 m (area a in Table 2); the rest were found about 1.5 km farther up the mountain within an area of about 0.5 km². The altitude of this site is approximately 1,240 m (19°8'N, 155°41'W). The entire region originally supported a rich rainforest, which was penetrated in 1926 by a single, massive, but narrow (200 m) tongue of lava, which terminated a few hundred meters farther down the slope, at about 1,220 m altitude. The geological action did not result in the formation of kipukas (patches of lower forest surrounded by a newer, higher lava flow) in this particular area. In 1965–1966, however, heavily forested areas not affected by the 1926 flow were bulldozed in order to create pasture. In the process, certain low pockets of dense vegetation were not cleared, because of locally rough terrain. Rubble from the cleared areas was pushed to the margins of these places, so that they formed small but dense man-made "ranch kipukas" (see photographs taken at Kahuku Ranch in Carson [1982]). Kipukas formed by lava flows in other areas of the island of Hawaii

resemble these man-made kipukas. The collections that yielded hybrids, begun in 1974, were made at the 1,160 m site and on both sides of the 1926 flow. Most of the hybrids were collected within the ranch kipukas.

Populations within each ranch kipuka were small. This appears to be due to the scarcity of sites available both for oviposition and for the formation of male leks. We theorize that one or possibly more of these kipukas could have sheltered a deme of critically small size for the efficient operation of mate choice, due to the reduction in the number of male individuals on the leks. This could especially apply to the rarer of the two sympatric species at the time. We note that, in the first four collections, *D. heteroneura* outnumbered *D. silvestris* by 5 to 1 (Table 2).

The situation described above resembles that of another *Drosophila* species pair, *D. ochrobasis* and *D. setosimentum,* which were also found hybridizing at higher altitudes (where *D. setosimentum* is the rarer species) in the Forest Reserve above the Ranch lands (Carson et al., 1974). In the Olaa Tract, where hybridization was also found, both *D. heteroneura* and *D. silvestris* had become very infrequent in collections at the time that the hybrid was found; neither species has been collected in this area of forest for the past seven years (Carson, 1986*b*).

Accordingly, we suggest that the reduction of a population to a series of local relicts may have forced an unusual situation, which might be expected to set the stage for a breakdown of the normal intraspecific female mate-choice system. We hypothesize that at least one *D. silvestris* female accepted a *D. heteroneura* male under such circumstances.

The hybridization events described here are interesting in view of the studies of mitochondrial DNA (mtDNA) of DeSalle et al. (1986) and DeSalle and Giddings (1986). In these studies, the mtDNA results suggest that hybridization has occurred in the past between several other pairs of closely related species in the *planitibia* subgroup of *Drosophila* as well as specifically between *D. silvestris* and *D. heteroneura.*

Whereas our observations indicate that the cross *D. silvestris* ♀ × *D. heteroneura* male has occurred, the data of DeSalle et al., (1986) record that several *D. silvestris* females showed mtDNAs that resemble *D. heteroneura* mtDNA. If hybridization is invoked to explain this similarity, it must have necessarily involved a *D. heteroneura* ♀ × *D. silvestris* ♂ hybridization, rather than the reciprocal cross observed by us. Based on laboratory experience with these species, however, there is no reason not to accept the hypothesis that both reciprocal crosses could occur in nature, especially if the relative abundance of the two sympatric species were reversed.

Morphological and color variation exists widely within populations of *D. silvestris,* both in areas where it is currently sympatric with *D. heteroneura* and in areas where the species are currently allopatric. As a species, *D. heteroneura* shows less variation. The extent to which the variability of *D. silvestris* is due to past hybridization between the species is not known. It is clear, however, that the variability in these other populations currently does not include the crucial characters of F_1 hybrid males (the color of the face and the width of the head). The data are suggestive that some of the variability within *D. silvestris* may be derived from past introgression from *D. heteroneura.*

In conclusion, we offer the following hypothesis for the evolution of *D. silvestris* and *D. heteroneura.* First, allopatric ancestral populations acquired, through sexual selection, the separate syndromes of characters that they now display. When partial sympatry occurred, sexual selection did not prevent cross-mating under all population conditions, perhaps because these two species are newly evolved. They appear to be at a stage in the evolution of sexual selection before the pleiotropic effects of gene differences have produced de facto reproductive isolation. The coherence of each gene pool is a much better basis for the characterization of a species than reproductive isolation, especially during periods of initial divergence of the populations.

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