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Chromosomal inversion patterning and population differentiation in a young insular species, *Drosophila silvestris*

(genetic polymorphism/hierarchical *F* statistics/Hawaiian *Drosophila* evolution/microgeographic variation)

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ABSTRACT **The recently evolved Hawaiian species *Drosophila silvestris* has a subdivided population structure and shows great spatial heterogeneity in chromosome inversion distributions and frequencies within its extremely limited geographic range. Pattern analysis of the 11 chromosomal polymorphisms in the context of the recently discovered morphological and behavioral divergence within the species has elucidated the history of the chromosomal differentiation. We identify four chronological groups of inversions and their probable sites of origin. Spread of the derived "3-row" bristle morphotype on the Hilo side of the Island of Hawaii has been accompanied by the acquisition of six new inversion polymorphisms. Three phylogenetically old inversions show correlations with altitude, and there are multiple cross-correlations between inversions on the same and different chromosomes, reflecting complex interaction systems. Quantification of the genetic population structure of *D. silvestris* by hierarchical *F* statistics reveals a dramatic level of genetic differentiation for an evolutionarily new species of such restricted range. This level exceeds that of older, continental *Drosophila* species. There is, however, minimal concordance between the chromosomal variation and the morphological–behavioral discontinuity, a consequence of the extensive cytological variation within each morphotype. Such a fragmented gene pool favors the rapid evolution and continued divergence of this insular species.**

The genetic structure of natural populations underlies both the potential path and the rate of evolutionary change in individual species. The *Drosophila* endemic to Hawaii are well known as the example *par excellence* of rapid speciation; over 700 species have evolved in recent geological time from a small number of ancestral founders reaching the isolated Hawaiian Archipelago (1, 2). Apart from qualitative observations of the microgeographic dissection and patchy nature of the habitats of these flies, as a result of volcanic factors and restricted host plant distributions (1–3), there have been no attempts to quantitatively analyze the population structure and patterns of gene flow within any species of Hawaiian *Drosophila*.

In recent years, population studies (4–9) have focused on *Drosophila silvestris*, a species endemic to the Island of Hawaii. Given that the oldest volcano on this still actively growing island is dated at less than 400,000 years (10), it can be inferred that *D. silvestris* is historically new; populations on the slopes of the newer volcanoes of the island are correspondingly younger. This species is chromosomally polymorphic for 11 paracentric inversions and displays marked interpopulation variability (5). Inversion polymorphism plays an important role in the population dynamics of many *Drosophila* species (11). The organization of blocks of genes into balanced coadapted complexes via such chromosomal arrangements may permit the evolution of genetic interaction systems and an enhancement of overall fitness. Such a process can be presumed to have taken place in *D. silvestris* as each successive new inversion arose and became incorporated into a local population.

Within the island, *D. silvestris* has been subdivided into two regionally disjunct sets of populations that show behavioral and morphological differences (8). To the west and south ("Kona side"), the populations give evidence of being phylogenetically older than those of the "Hilo side" to the north and east (7, 9). Here, we reexamine the spatial patterns of cytological differentiation within *D. silvestris* in relation to the recently discovered morphological discontinuity, in an attempt to understand the microevolutionary processes driving the divergence of this recently evolved species.

Using the chromosomal inversions as markers, we apply a genetic approach, namely hierarchical *F* statistics (12), to analyze population structure of this young species. We compare this structure with that found in ancient continental species in terms of extent of chromosomal differentiation, in order to assess the nature of the evolutionary events occurring in the early dynamic stages of establishment of an insular species.

MATERIALS AND METHODS

Specimens of *D. silvestris* were captured from 17 populations on the Island of Hawaii (Fig. 1), comprising 5 from the west and south regions of the island (sites 1–5, Kona side) and 12 from the north and east (sites 6–17, Hilo side). The sites are as follows: 1, Hualalai, 1100 m; 2, Hualalai, 1400 m; 3, Pauahi, 1372 m; 4, Kahuku Ranch, 1220 m; 5, Kipuka Pahipa, 1646 m; 6, Kohala, 1220 m; 7, Maulua, 1539 m; 8, Piihonua, 1341 m (8A, 1971–72 sample, referred to as Kipuka 4400′ in ref. 5; 8B and 8C, samples from 1976 and 1978 collections, respectively); 9, Mawae Kipuka 9, 1554 m (9A, 9B, and 9C, samples from 1967–68, 1971–72, and 1976 collections, respectively); 10, Mawae Kipuka 14, 1524 m; 11, Kilauea Forest, 1615 m; 12, Keauhou Ranch, 1433 m; 13, Upper Olaa, 1358 m; 14, Kulani Trail, 1328 m; 15, Volcano Experiment Station, 1237 m; 16, Olaa Tract, 1147 m; and 17, Rain Sheds, Keauhou Ranch, 1298 m. Further information on these localities is given in refs. 5 and 6.

The chromosomal inversion polymorphisms in each of these 17 populations were analyzed from F_1 larval polytene squash preparations as previously described (5). The comprehensive inversion data on *D. silvestris* presented and analyzed here include data published in refs. 5, 6, and 9, and more recent, previously unpublished information derived from collections of this species spanning a 20-year period from 1967 to 1987. All independent samples from the same locality were compared by heterogeneity *G* tests (G_H) performed on the frequencies of each individual inversion, and homogeneous samples were pooled. Pearson correlation coefficients between the altitudes of the 17 localities and the

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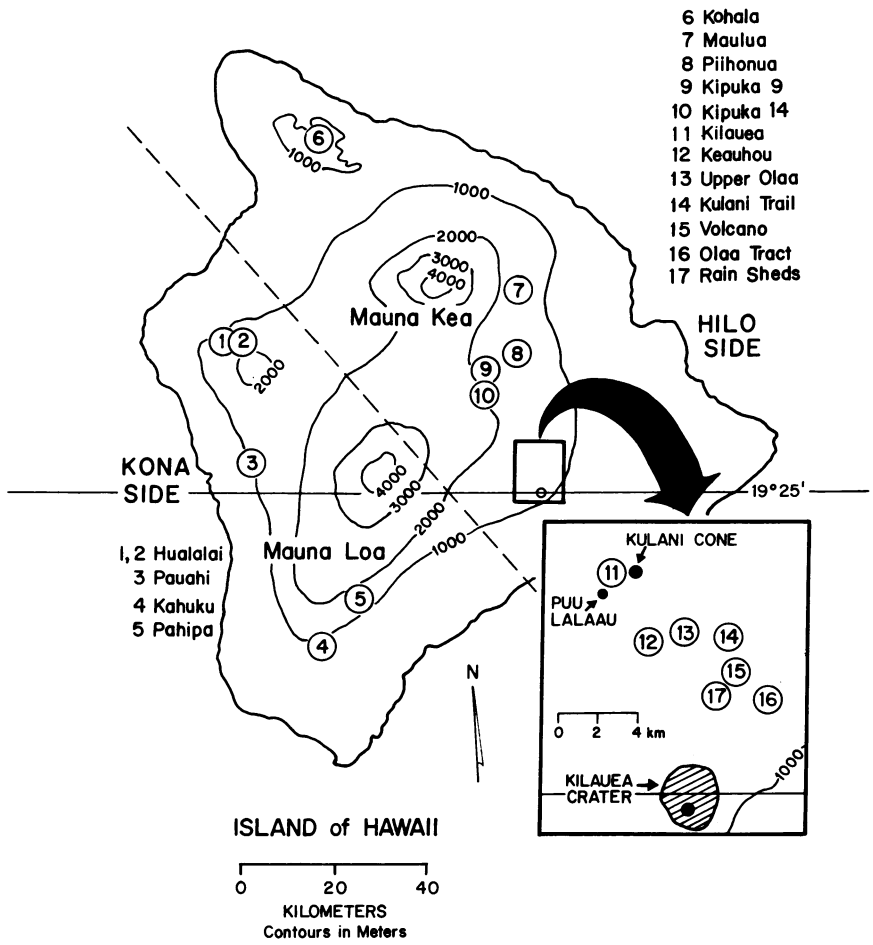

FIG. 1. Locations of the sampled *D. silvestris* populations or demes (D) on the Island of Hawaii. The broken line divides the total species distribution area (T) into two regions (R). Males from the phylogenetically older Kona-side populations (sites 1–5) have two rows of tibial cilia; males from Hilo-side populations (sites 6–17) have three rows.

frequencies of each inversion, and between frequencies of pairs of inversions, were computed by using the SPSS-X statistical package (13).

The population structure of *D. silvestris* with respect to its chromosomal arrangements was assessed by Wright's hierarchical *F* statistics (12). Populations sampled from each of the 17 sites were treated as panmictic demes (D), an assumption previously verified (14). For the demes at Piihonua and Kipuka 9, only the frequency data from the larger, contemporaneous 8A and 9B samples, respectively, were used in these analyses. The total population (T) was divided into two regions (R; see Fig. 1 caption), based on a morphological character, the number of rows of cilia on the male foreleg tibia, a secondary sexual character which provides a nonarbitrary marker for species subdivision (6). Using a subroutine from the Biosys-1 program (15) which corrects for the sampling variance (12), the fixation indices, F_{DT}, F_{RT}, and F_{DR}, were calculated for each individual inversion. These indices are related by the equation (12)

$$(1 - F_{DT}) = (1 - F_{DR})(1 - F_{RT})$$

where

$$F_{XT} = \sigma^2_{q(XT)}/[q_T(1 - q_T)],\ q_T = (1/n)\sum_{i=1}^{n} q_i,$$

and q_i is the inversion frequency in the ith population (see Table 1).

RESULTS

D. silvestris has five paracentric inversions in chromosome 4, and two in each of chromosomes 2, 3, and X. Table 1 summarizes the available inversion frequency data for 17 natural populations from which at least 50 sets of autosomes have been sampled. Individual populations are polymorphic for 4 to 11 inversions. Three inversions, 3m, $4k^2$, and 4t, are species-wide in their distribution. Others are more restricted (see also footnotes to Table 1), with two inversions, Xt^3 and $4p^3$, being endemic to just a single highly polymorphic population at Maulua (site 7). The older Kona-side populations in which males have two rows of bristles are less polymorphic cytologically than the "three-row" Hilo-side populations, lacking 6 of the 11 inversions. Within each region, the frequencies of each of the 5 and 11 polymorphic inversions, respectively, show significant heterogeneity among populations (heterogeneity $\chi^2_{4\ df} = 27.2 - 174.7$, $P < 0.0001$ for the Kona-side populations; heterogeneity $\chi^2_{11\ df} = 51.3 - 503.1$, $P < 0.00001$ for the Hilo-side populations).

Six populations for which successive samples were found to be homogeneous (sites 1, 3, 5, 6, 10, and 16) displayed temporal stability over time frames of 6–13 years. Temporal shifts in inversion frequencies were found, however, in two of the smaller Kipuka populations. At Piihonua (site 8, Table 1) significant shifts occurred in the frequencies of 3m and $4k^2$ (G_H = 10.3 and 9.7, respectively; 2 df, $P < 0.01$). In the Mawae Kipuka 9 population (site 9, Table 1), there were temporal shifts in the frequencies of all polymorphic inversions except 3m (six G_H values range from 6.1 to 12.4; 2 df,

Table 1. Population frequencies of *D. silvestris* inversions

Site no.	N^A	Inversion frequency 2o	3m	3r	$4k^2$	4t	$4l^2$	$4m^2$
				Kona-side populations				
1	76	—	0.816	0.067	0.566	0.776	—	—
2	188	—	0.495	0.005	0.356	0.707	0.048	—
3	231	—	0.684	0.004	0.641	0.784	—	—
4	293	—	0.894	—	0.761	0.802	0.010	—
5	108	—	0.370	—	0.213	0.565	0.306	—
	896							
				Hilo-side populations				
6	59	0.169	0.898	0.492	0.746	0.780	—	0.237
7*	118	0.059	0.373	0.102	0.415	0.661	0.331	0.212
8A†	51	—	0.941	0.529	0.863	0.640	0.039	0.255
8B†	20	—	0.750	0.300	0.500	0.500	0.050	0.200
8C†	28	—	1.000	0.286	0.750	0.536	0.071	0.107
9A	110	0.009	0.436	0.055	0.309	0.845	0.155	0.109
9B	176	0.045	0.551	0.142	0.398	0.710	0.261	0.085
9C	30	0.167	0.567	—	0.600	0.800	0.067	—
10	187	0.112	0.561	0.059	0.406	0.840	0.021	0.070
11	436	0.028	0.324	—	0.511	0.782	0.179	0.016
12	88	—	0.625	0.023	0.693	0.943	0.011	0.011
13	372	0.011	0.745	—	0.796	0.970	0.027	—
14	54	—	0.815	—	0.685	0.963	0.019	—
15	194	—	0.954	—	0.851	0.974	—	—
16	106	—	0.925	0.039	0.830	0.840	—	—
17	67	0.030	0.507	0.030	0.612	0.866	0.060	—
	2096							

N^A = no. of autosomes scored.
*Additional inversions: 2t, 0.025; $4p^3$, 0.136; Xo^3, 0.025; Xt^3, 0.089 (no. of X chromosomes, N^X, = 79).
†Additional inversions, in 8A, 8B, and 8C, respectively: 2t, 0, 0, 0.036; Xo^3, 0, 0.059, 0.042 (N^X = 29, 17, 24).

$P < 0.05$). Some inversions, 2o and $4k^2$, for example, appeared to show directional changes over the 9 years between initial and final samples; others simply fluctuated in frequency.

Some of the significant correlations between altitude and inversion frequencies, and between inversions, are presented in Table 2. Not shown are 10 additional significant correlations of the rare and geographically restricted inversions Xt^3, Xo^3, 2t, and $4p^3$, which correlate with each other and, in some instances, with the moderately low frequency inversions 3r, $4l^2$, and $4m^2$. Three inversions show strong correlations with altitude, that for $4l^2$ being positive, whereas those for 3m and $4k^2$ are negative. As expected, these three inversions show significant correlations with each other. There are additional significant intrachromosomal correlations among the several fourth chromosome inversions—i.e., between $4k^2$ and 4t, 4t and $4l^2$, and $4p^3$ and $4l^2$. More unexpected are the correlations between the frequencies of some of the more common inversions on independent chromosomes, such as those between 2o and 3r, and between 3r and $4m^2$.

Table 3 presents the hierarchical fixation indices F_{DR}, F_{RT}, and F_{DT} for the 11 individual inversions, along with the species mean frequency, q_T, for each inversion and the weighted mean value of each index. Some 84% of the total chromosomal diversity in the species is within demes. The population structure differs among individual inversions, with the greatest differentiation among the 17 demes (cf. F_{DT} values) being shown by the third chromosome inversions 3r and 3m. A striking feature of this set of hierarchical F values is that, contrary to expectation, the F_{DR} values are generally somewhat greater than the F_{DT} values, leading to negative F_{RT} values; this indicates a lack of clear chromosomal differentiation between the Kona-side and Hilo-side populations of the species. Although the individual F values for the 2o, $4k^2$, 4t, and $4m^2$ inversions depart from this pattern, the F_{RT} values are minimal, suggesting only marginal differentiation between regions for these four inversions.

Table 2. Partial matrix of the significant ($P < 0.05$, df = 15) product-moment correlation coefficients among the population frequencies of the common *D. silvestris* inversions and altitude

	3m	3r	$4k^2$	4t	$4l^2$	$4m^2$
Alt.	−0.835**		−0.715*		0.759**	
2o		0.505				0.592
3m			0.845**		−0.736**	
3r						0.885**
$4k^2$				0.583	−0.705*	
4t					−0.639*	

*, $P < 0.01$; **, $P < 0.001$ for the two-tailed test of significance.

Table 3. Population structure of *D. silvestris* chromosomal inversions measured by hierarchical F statistics

Inversion	q_T	F_{DR}	F_{RT}	F_{DT}
Xo^3	0.004 ± 0.003	0.019	−0.002	0.017
Xt^3	0.005 ± 0.005	0.084	−0.009	0.076
2o	0.027 ± 0.012	0.071	0.002	0.073
2t	0.004 ± 0.002	0.016	−0.002	0.014
3m	0.675 ± 0.053	0.215	−0.026	0.195
3r	0.088 ± 0.040	0.316	−0.011	0.308
$4k^2$	0.608 ± 0.048	0.145	0.000	0.145
4t	0.800 ± 0.029	0.073	0.005	0.077
$4p^3$	0.008 ± 0.008	0.133	−0.013	0.122
$4l^2$	0.077 ± 0.028	0.187	−0.023	0.168
$4m^2$	0.052 ± 0.022	0.143	0.005	0.147
$\bar{F}_{XY}$		0.165	−0.009	0.158

q_T = mean ± SEM inversion frequency across all populations.

DISCUSSION

The evolutionarily young species *D. silvestris* displays marked chromosomal heterogeneity between populations, with no two populations being alike with respect to their inversion composition and frequencies. The present distribution of this insular species is limited to less than 700 km^2 by the occurrence of suitable forest habitat at elevations of 1100 to 1650 m, the local rarity of some of its host plants within this range, and dissection of the habitat by lava flows and forest clearing activities of humans. The patchy nature of its geographic distribution within five largely disjunct areas on the Big Island of Hawaii would suggest that the species has a fragmented gene pool. The degree of population subdivision has evidently been conducive to the establishment of novel morphological and behavioral traits in parts of the species range (6–8), leading to a distinct discontinuity between the Kona-side and Hilo-side regions of the species distribution. This analysis uses the unique attributes of inversion polymorphisms to elucidate the genetic population structure and history of *D. silvestris*, and thus the early dynamic phases of the evolutionary differentiation of species in the insular situation.

It is commonly accepted that chromosomal arrangements, such as inversions, have a unique origin (16), by contrast with electrophoretic or other alleles. The subsequent widespread establishment of an inversion polymorphism is therefore contingent upon selection favoring the new arrangement in some way, often because of local restrictions upon recombination imposed when in the heterozygous condition. The altitudinal correlates of 3m, $4k^2$, and $4l^2$ frequencies (Table 2) substantiate the adaptive properties of these arrangements and the likely operation of selection in maintaining these particular polymorphisms. It seems significant that the three

inversions involved in elevational clines are among the oldest inversions in the species, whereas no such clines exist for any of the historically newer inversions which are restricted to the geologically younger Hilo side of the species range.

Actually, all 11 *D. silvestris* inversions are "new"; none are found in the monomorphic, ancestral species from Maui (2, 5). The distributional data suggest the following reconstruction of the evolutionary history of chromosomal differentiation in *D. silvestris*, with the 11 inversions ordered into four groups in terms of their probable age. The most ancient inversions in the species are undoubtedly 3m, 4t, and $4k^2$, which have a species-wide distribution and must have arisen in the ancestral *D. silvestris* population with two rows of tibial cilia, most likely in the Hualalai region (7). Next to arise may have been $4l^2$ and 3r, found in 12 and 11 populations, respectively, from both sides of the island. The 2o and $4m^2$ inversions constitute the third group, currently found in 8 and 7 Hilo-side populations, all with three rows of cilia. These two inversions both have their maximum frequencies in the Kohala population (site 6, Fig. 1), and it can be postulated that they arose there, in conjunction with the major genetic shift that led to the novel "three-row" phenotype and associated mating behavior modifications. Once established in Kohala, founders with the derived morphological and behavioral phenotype dispersed southeastwards, successively establishing populations on the newer volcanoes and carrying the cluster of seven inversion polymorphisms with them. More recently, most likely in the Maulua population, four new inversions ($4p^3$, Xt^3, Xo^3, and 2t) arose; this youngest group of low-frequency inversions is restricted to one or two "three-row" populations on the Hilo side. This general scenario of the sequential cytological differentiation of *D. silvestris* populations inferred from the pattern of inversion polymorphisms coincides with that deduced from behavioral (7), morphological (9), and mitochondrial DNA (17) data. The founding of the Kohala population seems to have been followed by a veritable genetic revolution, with the episode of sexual selection which destabilized the mating behavior system being accompanied by a burst of chromosome breakage, ultimately leading to six new inversion polymorphisms.

The acquisition of each new inversion polymorphism in the relatively brief history of this species must have entailed the evolution of a new superior interaction system, the outcome of selection primarily for coadaptation among loci within each arrangement to make it adaptive, as well as coadaptation between alternative arrangements to form a vigorous heterozygote (11, 12). For the older inversions, there is evidence both of their adaptive nature with respect to an environmental parameter, altitude, and of considerable heterosis between arrangements, particularly in the case of the three chromosome 4 arrangements, k^2, t, and l^2 (18). Also important is selection for coadaptation with other components of the genome, notably existing inversions on the same and different chromosomes. Some initial linkage disequilibrium between inversions would be predicted when a new inversion arises on a chromosome already carrying one or more inversions. Indeed, the disequilibrium on chromosomes 3 and 4 persists (5). The correlations between chromosome 4 inversions, and between inversions on independent chromosomes (Table 2) further attest to the complex interactions involved in the process of achieving a new and balanced genetic system. This phase of the selective process can be assumed to be still occurring in the case of the four newest inversions, as the shifting balance process of evolution (12, 19) continues to operate within and among *D. silvestris* populations.

Because each inversion has a unique origin, these arrangements are particularly valuable as sensitive indicators of gene flow. The rare and more recent inversions Xo^3 and 2t shared by the Maulua and Piihonua populations (sites 7 and 8, Table 1, Fig. 1) substantiate genetic contact between these two populations, which are 20 km apart. Nonetheless, such gene flow must be currently limited since the slightly more frequent inversions Xt^3 and $4p^3$ have not spread from Maulua to Piihonua, and none of these four have spread to the Mawae Kipukas 9 and 14, 25 km distant from Maulua. These two Mawae Kipukas (sites 9 and 10, Fig. 1, Table 1), separated by bare lava, are only 1300 m apart, yet populations show extensive genetic differentiation (5). Moreover, the Kipuka 9 population has shown temporal shifts in inversion frequencies while the one in Kipuka 14 has been temporally stable over part of the same time period, suggesting their independent evolution. Together, these observations imply strong isolation between current *D. silvestris* populations. Past gene flow presumably linked contiguous populations or those having an ancestor–founder relationship. The extinction of intermediate populations by volcanic lava flows interrupted gene flow, in some cases reducing it almost to nil.

Hierarchical *F* statistics applied to the chromosome polymorphism data from *D. silvestris* validate its subdivided population structure. The average F_{DT} value of 0.158 combined across inversions (Table 3) indicates moderately great local differentiation among populations (12), with some 16% of the total genetic variance being thus distributed. Essentially all of this diversity is due to that between demes within regions ($F_{DR} = 0.165$), with a negligible component of diversity due to differentiation between the two regions. [The slightly negative F_{RT} value is indistinguishable from zero. The problem of negative variance components resulting from the partitioning of the estimated total variance is one which is accentuated in Wright's procedure (15)]. Thus, the current subdivision of *D. silvestris* into morphologically and behaviorally distinct Kona-side and Hilo-side populations does not directly coincide with the chromosomal inversion patterning. This apparent anomaly perhaps results from the fact that the several rare inversions, like rare alleles, have little effect on statistical measures of genetic diversity, whereas the more common inversions have equivalently broad ranges of frequency variation within each of the two regions. The differences in hierarchical pattern between individual inversions can be partially attributed to differential selection, but more significantly to historical factors. In general, the older inversions show higher *F* values and greater differentiation, as expected as a function of the greater time since their origin, but there are exceptions—e.g., 4t.

The level of population differentiation in this young, insular species might be predicted to be less than that in more ancient, continental *Drosophila* species. In *D. pseudoobscura*, F_{DT} based on chromosome 3 arrangement polymorphisms in the western United States is 0.273 (12), and in the European species *D. subobscura*, F_{DT} averaged across all five chromosomes is 0.306 (20). The great disparity in geographic areas, however, impedes making a direct comparison of these *F* values. The distribution areas of *D. pseudoobscura* and *D. subobscura* are, respectively, over 5000 and 2000 times that of *D. silvestris*. Restricting consideration to a geographic region, a mean F_{DR} value of 0.165 in *D. silvestris* compares with values of 0.075 and 0.097 in *D. pseudoobscura* and *D. subobscura*, respectively. But regions for these two latter species comprise areas that are over 2000 and 400 times the average area of a region for *D. silvestris* (350 km^2). This insular species therefore shows significantly greater population differentiation than the continental species. Given its extremely limited distribution and its relative youth, the fine-grained nature of the differentiation in *D. silvestris* is astounding.

The microgeographic variation in chromosomal inversion frequencies in *D. silvestris* reflects its past history, notably the stepwise founder origins of populations (3), and its current subdivision into a number of isolated demes. This

fragmentation of the gene pool has already led to the evolution of a novel morphotype, perhaps an incipient species (6, 8), and sets the stage for the continued rapid evolution of this recently evolved Hawaiian *Drosophila* species.

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