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Evolution

Discordance of nuclear and mitochondrial DNA phylogenies in Hawaiian *Drosophila*

(planitibia subgroup/founder principle/hybridization)

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ABSTRACT ***Drosophila differens*, endemic to Molokai, *Drosophila planitibia* of Maui, and *Drosophila silvestris* and *Drosophila heteroneura* from the island of Hawaii are chromosomally homosequential species that presumably have colonized the newer islands of the Hawaiian archipelago by sequential founder events. We have examined the phylogenetic relationships of these four species by using mitochondrial DNA restriction site data for 23 enzymes. Both distance and character-state analyses indicate that a sequential or monotonic branching relationship exists for mtDNA restriction site data from the four species. The mtDNA data suggest that the maternal lineage that gave rise to *D. differens* is ancestral to the *D. planitibia* maternal lineage, which in turn shares the most recent common ancestor with the *D. silvestris* and *D. heteroneura* maternal lineages [with *Drosophila hemipeza* (Oahu) and *Drosophila neopicta* (Molokai and Maui) as outside references]. We also discuss the phylogenetic implications of the mtDNA data in comparison with other sources of phylogenetic data. We conclude that hybridization of the species in this group has been an important factor in the evolution of the nuclear genomes. Because of small population sizes and mating asymmetries, it is possible that the nuclear genetic distance of species that are physically capable of hybridizing (e.g., on the same island or island complex) is depressed. Consequently the mtDNA genetic distance appears to be more sensitive in establishing the sequence of evolutionary events responsible for the present distribution and population structure of these species.**

The 16 species comprising the planitibia subgroup of Hawaiian *Drosophila* reside in moderate altitude rainforests on five of the six major high islands in the Hawaiian archipelago. Founder events have been suggested to play a major role in the evolution of these flies. Because of the sequential geological formation of islands in the Hawaiian chain (1–3), founder events also appear to have occurred in a sequential pattern from the older (northwest) to the younger (southeast) islands. The current high islands of the Hawaiian archipelago relevant to this study and their approximate ages, in megayears (million years; Myr), are Oahu (3.5 Myr), Molokai (1.5 Myr), Maui (1.0 Myr), and Hawaii (0.4 Myr). Occasional back-migrations from younger to older islands have been suggested (4).

Drosophila differens of Molokai, *Drosophila planitibia* of Maui, and both *Drosophila silvestris* and *Drosophila heteroneura* of the island of Hawaii are a cluster of four chromosomally homosequential species from the planitibia subgroup that have presumably colonized the islands of the Hawaiian archipelago by sequential founder events. Molokai is thought to be the center of the planitibia subgroup radiation (5). These four species are differentiated chromosomally from other of the planitibia subgroup flies by having a unique polytene inversion designated "*Xr*" (1, 5–7).

Several classical evolutionary approaches (discussed below) have been taken to examine the phylogeny of these four closely related species. In particular, the identification of the putative founders of *D. heteroneura* and *D. silvestris* has been attempted (1, 3, 8–10). A novel, possibly more sensitive approach to the analysis of these organisms is the examination of DNA sequences with restriction endonucleases. The development of statistical tests for the analysis of phylogenies based on restriction sites and DNA sequence data (11–13) also makes this approach attractive.

For a number of reasons, mtDNA appears to be a suitable choice to examine the phylogeny of these organisms. First, mtDNA evolves at a rate suited to the study of groups whose divergence times are no greater than from 8 to 10 Myr (14). The four species in this study are most likely to have diverged within the last 2 Myr (15). Second, the sensitivity of certain DNA sequences to stochastic changes should also be considered. Much of the evolution of this subgroup is thought to have occurred via founder/flush cycles, with the founders being perhaps so few as single gravid females. Therefore, a maternal marker might be sensitive in tracing ancestral relationships among these species. mtDNA is maternally inherited in these species (16) and should serve as an excellent marker of maternal lineages.

To examine properly the genetic and phylogenetic relationships of these four homosequential species' mtDNA, we have included two related species as outside references. *Drosophila hemipeza* is the closest relative to the four homosequential species mentioned above on the basis of polytene chromosome relationships (4). [*D. hemipeza* has an unique inversion (Xc^2) and lacks the inversion found exclusively in the four taxa mentioned above.] We include *Drosophila neopicta* of Molokai and Maui, also a member of the planitibia subgroup, as an outgroup in this study because it carries three chromosomal arrangements as polymorphisms with the newer, inverted gene orders. These polymorphisms are presumed to be ancient because they also are found in related species restricted to two older islands (17).

MATERIALS AND METHODS

DNA manipulation and restriction site mapping were as described (18). The 23 restriction enzymes used are identical to those in ref. 19. Complete restriction site maps for all individual flies examined in this study have been recorded

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Abbreviations: Myr, megayears (million years); UPGMA, unweighted pair grouping using arithmetic averaging.

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Table 1. Phylogenetically informative restriction site states for the nine mtDNA haplotypes used in this study

	SH	SK	HK	HH	Pl	Di	Hz	N1	N2	Map position
a1	1	1	1	1	1	0	0	1	1	3.0
a3	1	1	0	0	1	0	0	0	0	4.9
a5	1	1	1	1	1	0	0	0	0	8.6
a9	0	0	0	0	1	1	1	1	1	13.6
a11	0	0	0	0	1	1	0	0	0	15.2
a13	0	0	0	0	0	1	1	1	1	4.4
d2	1	1	1	1	0	0	0	0	0	1.4
d3	1	1	1	1	0	0	0	0	0	1.7
d4	1	1	1	1	0	0	0	0	0	3.5
d6	1	1	1	1	0	0	0	0	0	12.7
d10	0	0	0	0	0	1	1	1	1	16.0
f2	0	1	1	1	1	1	0	1	1	10.1
f4	0	0	0	0	1	0	1	0	0	2.4
g2	0	0	1	0	1	0	0	0	0	0.5
g4	1	1	1	1	1	0	1	0	0	5.7
g7	0	1	0	1	1	1	0	0	0	13.9
g8	1	1	0	1	1	1	0	1	1	14.6
g9	1	1	1	1	1	1	0	0	0	15.4
g10	1	1	1	1	1	0	1	0	0	15.9
g11	0	0	0	0	0	0	1	1	1	7.6
g12	0	0	0	0	0	0	0	1	1	8.2
g15	0	0	0	0	0	1	0	1	0	9.4
g14	0	0	0	0	0	0	0	1	1	10.1
i1	1	1	1	1	1	0	0	0	0	12.4
i2	0	0	0	0	1	1	0	0	0	4.1
i3	0	0	0	0	0	0	0	1	1	11.6
c6	0	0	0	0	0	1	1	0	0	13.7
e1	1	1	1	1	0	0	0	0	0	0.7
e5	0	0	0	0	0	1	1	1	1	13.3
j3	1	1	0	1	1	0	0	0	0	11.1
j9	0	0	0	0	0	0	0	1	1	7.4
h1	0	0	0	0	1	1	1	1	1	2.2
h3	1	1	1	1	0	0	1	0	0	3.5
h7	1	1	0	1	0	1	1	0	0	10.8
h10	1	1	1	1	1	1	1	0	0	13.2
h11	0	0	0	0	0	0	0	1	1	4.0
m2	1	1	1	1	1	0	1	0	0	5.8
m4	1	1	1	1	0	1	1	0	0	8.1
k1	0	0	1	1	0	0	0	0	0	8.5
k2	0	1	0	0	0	1	0	0	0	16.0
k3	1	1	1	1	1	1	0	0	0	16.6
o4	1	0	0	0	1	1	0	1	1	9.5
o5	1	1	1	1	1	0	1	0	0	16.5
q1	0	0	0	0	1	1	0	1	1	12.0
p3	1	1	1	1	1	1	1	0	0	10.7
p5	1	1	1	1	1	1	1	0	0	15.1
p6	1	1	1	1	1	0	0	0	0	16.1
p10	0	0	0	0	0	0	0	1	1	8.7
p11	0	0	0	0	1	1	0	0	0	12.5
s5	0	0	0	0	0	1	1	0	0	13.2
s6	0	0	0	0	0	1	1	1	1	15.6
r2	1	1	1	1	1	0	0	0	0	6.4
r5	0	0	0	0	0	1	1	0	0	10.0
t1	0	1	1	1	0	0	0	0	0	8.6
u2	1	1	1	1	0	0	0	0	0	5.5
u3	0	1	0	1	1	1	1	0	0	5.5
v3	1	1	1	1	1	1	0	0	0	6.3
v6	0	0	0	0	0	0	0	1	1	14.5
x1	1	1	1	1	0	0	0	0	0	13.2
x2	0	0	0	0	0	0	0	1	1	12.6
w4	0	0	0	0	0	0	0	1	1	1.3

Zero and one indicate the absence and presence, respectively, of the restriction sites. Taxa are as follows: SH, *D. silvestris* from the Hilo side of Hawaii; SK, *D. silvestris* from the Kona side of Hawaii; HK, *D. heteroneura* from the Kona side of Hawaii; HH, *D. heteroneura* from the Hilo side of Hawaii; Pl, *D. planitibia* isoline U84Y from Waikamoi, Maui; Di, *D. differens* isoline U43V1 from Hanalilolilo, Molokai; Hz, *D. hemipeza* isoline W33J from Palikea, Oahu; N1, *D. neopicta* male W39BA from Hanalilolilo, Molokai; and N2, *D. neopicta* male W38BT from West Maui. Restriction endonucleases are as follows: a, *Ava* II; b, *Bam*HI; c, *Cla* I; d, *Bcl* I; e, *Eco*RI; f, *Bst*EII; g, *Bst*NI; h, *Hin*dIII; i, *Bst*XI; j, *Eco*RV; k, *Kpn* I; *m*, *Hpa* I; *n*, *Nru* I; *o*, *Nco* I; p, *Pvu* II; q, *Pst* I; r, *Sac* II; s, *Sac* I; t, *Sma* I; u, *Stu* I; v, *Sba* I; w, *Xmn* I; and x, *Xho* I. Map position is given in kb relative to the *Nru* site (which comprises the first two codons of the cytochrome oxidase I gene) going toward the mitochondrial genes encoding RNA (see ref. 16).

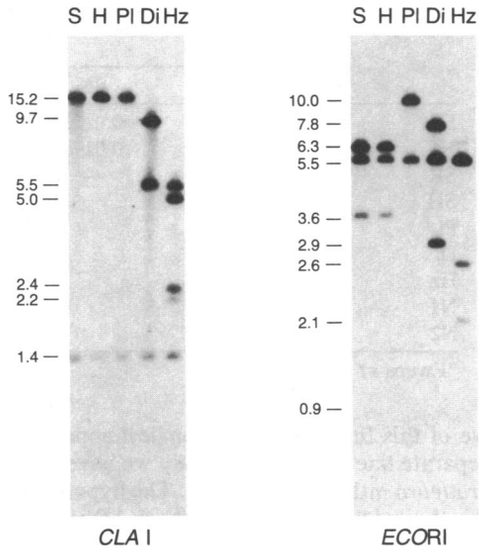

FIG. 1. Restriction fragment patterns of species in this study for the restriction endonucleases *Cla* I (*Left*) and *Eco*RI (*Right*). H, *D. heteroneura* from the Hilo-side of Hawaii; S, *D. silvestris* from the Hilo-side of Hawaii; P, *D. planitibia* isoline U84Y from Waikamoi, Maui; Di, *D. differens* isoline U43V1 from Hanalilolilo, Molokai; Hz, *D. hemipeza* isoline W33J from Palikea, Oahu.

(16) or are available on request from the authors. Procedures for phylogeny estimation and the testing of hypotheses on phylogenetic relationships have also been described (12, 19).

To facilitate phylogenetic analysis of restriction site data, we have inferred the hypothetical ancestors of closely related individuals within a species by using two *D. neopicta* individuals from Molokai as an outgroup. We used the inferred hypothetical ancestors of three *D. hemipeza* individuals from Palikea Ridge, Oahu, four *D. planitibia* individuals from Waikamoi, Maui, and the two *D. neopicta* individuals from Kipahulu Valley, Maui. Only one isoline of *D. differens* from Hanalilolilo, Molokai, was examined.

Phylogenetic relationships among populations of *D. heteroneura* and *D. silvestris* from the island of Hawaii have been discussed elsewhere (19). The results of that study indicate that there are at least three separate mtDNA lineages in these two species and that two distinct *D. silvestris* mtDNA lineages exist. One is found on the Kona side (SW) of the island of Hawaii and is accompanied by a morphological marker, the presence of two rows of tactile cilia on the tibia of the male. We include the hypothetical ancestor for the Kona-side population at Hualalai ($n = 4$) to represent this lineage. The second *D. silvestris* lineage is found on the Hilo side (NE) of Hawaii and is signaled by the presence of a third, irregular row of cilia between the other two on the tibia of the male. The importance of the differences in cilia row number is discussed in detail elsewhere (9, 20–22). We include the hypothetical ancestor for all of the Hilo-side *D. silvestris* (except the single isoline from Maulua, $n = 9$) as a repre-

Table 2. Distance matrix for the nine taxa used in this study

	Genetic distance data, *p* measure*								
	HH	HK	SK	SH	Pl	Di	Hz	N1	N2
HH	—	0.0156	0.0162	0.0254	0.0418	0.0575	0.0575	0.0664	0.0676
HK		—	0.0196	0.0237	0.0431	0.0606	0.0573	0.0669	0.0681
SK			—	0.0215	0.0365	0.0566	0.0580	0.0669	0.0680
SH				—	0.0339	0.0609	0.0594	0.0669	0.0680
Pl					—	0.0478	0.0575	0.0605	0.0620
Di						—	0.0492	0.0566	0.0533
Hz							—	0.0597	0.0597
N1								—	0.0223
N2									—

*Ewens *et al.* (23).

sentative of this lineage. *D. heteroneura* appears to form a third, separate lineage. For this study we have included two *D. heteroneura* mtDNA haplotypes. The hypothetical ancestor of the Waihaka *D. heteroneura* (n = 13) is included as a representative of the Kona side *D. heteroneura*, and the *D. heteroneura* isoline from Olaa (Q71G12) is included as a representative of the Hilo-side *D. heteroneura*. All *D. silvestris* and *D. heteroneura* hypothetical ancestors were inferred by using *D. neopicta* from Molokai as an outgroup.

RESULTS AND DISCUSSION

Restriction Site Analysis. The restriction fragment patterns shown in Fig. 1 are two typical examples of the many restriction site polymorphisms that we observed among these species. Each restriction site was mapped relative to the others by using the standard mapping techniques of double digestion and probing with specific cloned fragments of the mtDNA molecule. Table 1 shows the restriction site mapping data for the phylogenetically informative sites in the six species examined in this study.

Phylogeny Estimation. Table 2 gives genetic distance data for the nine taxa in Table 1, using the Ewens *et al.* (23) *p* measure, which estimates the percent divergence between any two mtDNA haplotypes. The results of unweighted pair grouping using arithmetic-averaging (UPGMA) cluster analysis (24) of these data are shown in Fig. 2. Phenetic relationships obtained from mtDNA data differ from those obtained with isozyme data (25, 26) and DNA reassociation data (8) with respect to the clustering of *D. differens* with *D. planitibia*. In both the isozyme analysis and the DNA reassociation study, *D. differens* clusters with *D. planitibia*.

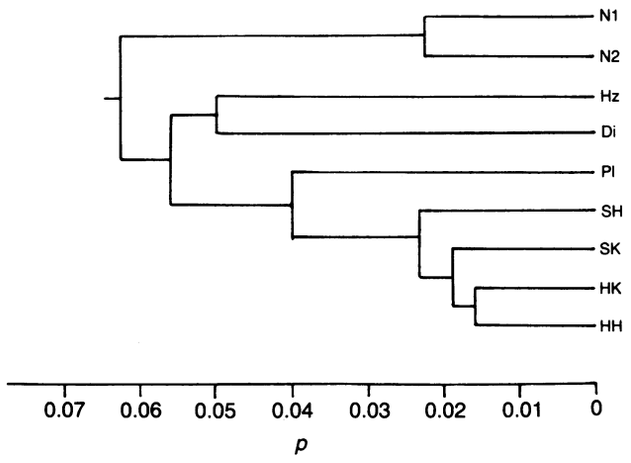

FIG. 2. UPGMA phenogram for Ewens *et al.* (23) *p* estimator from Table 3. Abbreviations are as in Table 1.

UPGMA analysis of the mtDNA distance data produces no such clustering.

Phylogenetic analysis using parsimony gave only one maximum parsimony topology for this data set (Fig. 3). Phylogeny MI suggests a stepwise or sequential branching of these species. Furthermore, the maximum parsimony topology is similar to the clustering relationships revealed by UPGMA (Fig. 2).

Hypothesis Testing. There is no assurance *a priori* that the maximum parsimony topology is more probable than other topologies. The application of Templeton's phylogeny hypothesis-testing algorithm (12) affords the opportunity to test alternative hypotheses and to assign probabilities to such alternatives. Several hypotheses on the origin and phylogeny of the species compared in this study have been proposed. These are based on behavior, morphology, biogeography, and genetic studies using isozymes, chromosome inversions, and DNA reassociation kinetics.

On the basis of morphological (1) and behavioral data, Kaneshiro (10) proposed that *D. differens* gave rise to *D. heteroneura* and that *D. planitibia* gave rise to *D. silvestris*. Phylogeny KI (Fig. 4) shows the topology that Hunt and Carson (8) attribute to Kaneshiro (10). Phylogeny KII is a modification of the initial hypothesis for the origin of *D. silvestris* and *D. heteroneura*. This topology was also suggested by Kaneshiro (10). A third topology, also consistent with Kaneshiro's proposal, is shown as KIII in Fig. 4.

On the basis of morphology, behavior, and biogeography, Spieth (2) suggests that "D. silvestris and *D. heteroneura* are the descendants of a migrant from the *D. differens* population of Molokai." In certain aspects this hypothesis is similar to that of Kaneshiro (10), however Spieth's conclusion might suggest that phylogenies SI or SII are plausible. The suggestion that *D. differens* is the closest relative of *D. silvestris* and *D. heteroneura* can be tested with phylogeny SIII, which places it in a position between *D. planitibia* and the *D. silvestris–D. heteroneura* branch.

Carson and Yoon (5) have suggested that phylogeny CI is correct on the basis of chromosome inversion data. Phylogeny CI implies that "a major burst of evolution" occurred that included the fixation of the *Xr* inversion in an

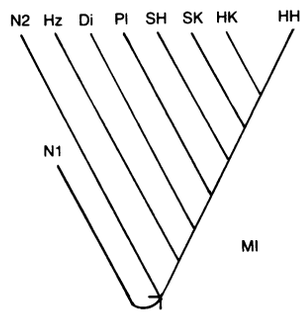

FIG. 3. Maximum parsimony topology (phylogeny MI) generated from the data listed in Table 1. Abbreviations are as in Table 1.

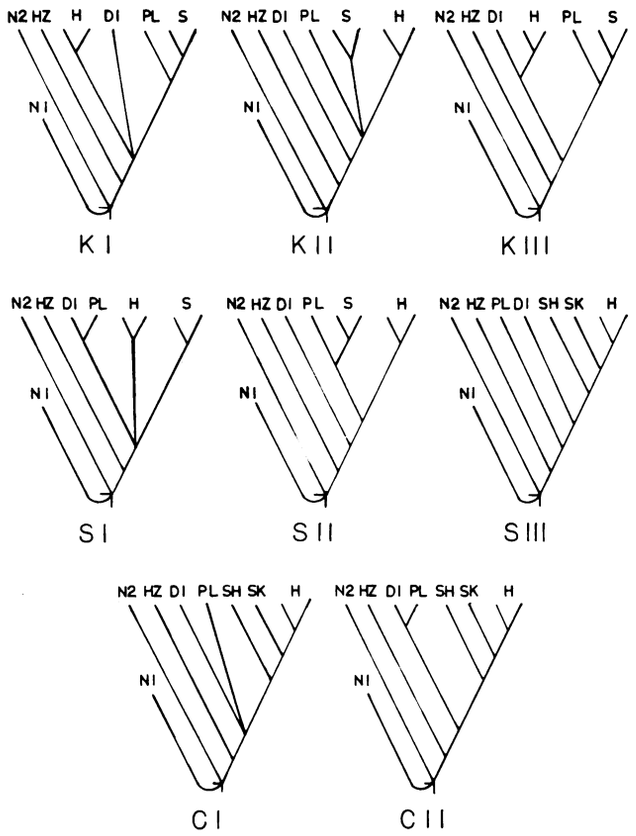

FIG. 4. Eight plausible alternative phylogenies (Phylogenies SI, SII, SIII, KI, KII, KIII, CI, and CII) consistent with previous phylogenetic studies of these species (using other methods such as morphology, behavior, isozymes, DNA reassociation kinetics, and chromosome inversion data). For an explanation of each topology see the text. Abbreviations are as in Table 1.

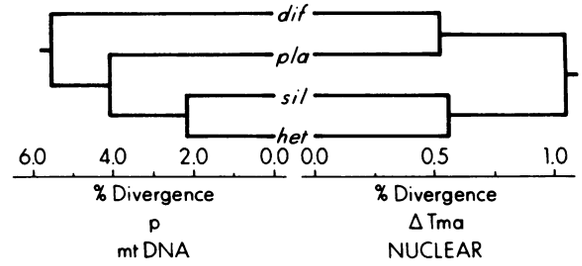

FIG. 5. Phenograms for the four homosequential species analyzed in this study showing the discordance between mtDNA and nuclear DNA branching orders. Percent divergence scales are for mtDNA (p, which is the percent divergence estimated from restriction site changes) from Fig. 2 and for nuclear DNA (T_{ma}, which gives the percent divergence from nuclear DNA reassociation studies) from ref. 8. dif, *D. differens*; pla, *D. planitibia*; sil, *D. silvestris*; het, *D. heteroneura*.

ancestral population on Molokai. The four homosequential species of this complex then evolved from this common ancestral population. Since *D. silvestris* and *D. heteroneura* share a polymorphic inversion (*3m/+*), Carson and Yoon (5) have suggested a close phylogenetic relationship between these two species. On the basis of isozyme analysis and single-copy DNA reassociation data, Hunt and Carson (8) (Fig. 3) have proposed phylogeny CII. This phylogeny is similar to CI and is also consistent with the chromosomal data.

The results of applying Templeton's algorithm in tests of phylogeny MI with all of the hypothesized phylogenies in Fig. 4 are shown in Table 3. The superiority of phylogeny MI to any of the eight other hypothesized phylogenies in Fig. 4 is highly significant. These results suggest that the phylogenetic relationships based on mtDNA data for the four homosequential species are best represented by the topology shown as MI in Fig. 3.

Phylogenetic Considerations. It is obvious from the large number of hypotheses on the origin and phylogenetic relationships of these species that no single phylogeny is likely to be entirely satisfactory in explaining all of the data. However, the existing chromosomal data are consistent with phylogeny MI. The *Xr* inversion could have been fixed on the branch between *D. hemipeza* and *D. differens*, and the polymorphic *3m/+* inversion could have occurred on the branch leading to the *D. heteroneura* and *D. silvestris* lineages. Phylogeny MI is partially consistent with the courtship behavioral hypothesis of Kaneshiro (10), which states that females from derived populations will mate preferentially with males from ancestral populations, while the converse is not true. The phylogenetic scheme proposed by phylogeny MI is in agreement with the data collected by Kaneshiro for four of the five species examined in this study. More recently. Hunt *et al.* (27) have compared the cytological location of a middle repetitive nuclear DNA sequence to examine the evolutionary relationships among the four chromosomally homosequential species. Their results suggest that *D. differens* is ancestral to *D. planitibia*, which in turn is ancestral to *D. silvestris* and *D. heteroneura*. This observation is entirely consistent with the maximum parsimony analysis of mtDNA restriction sites. But the best mtDNA phylogeny (MI) poses some inconsistencies with other available data.

The morphological traits described by Carson and Kaneshiro (1) in support of the idea that *D. differens* gave rise to *D. heteroneura* and that *D. planitibia* gave rise to *D. silvestris* are differences in pigmentation in the face and in the costal margin of the wing. These traits are extremely important in the courtship behavior of these flies. Individuals of both sexes have acute vision (2), and their courtship ritual relies heavily on visual cues. Therefore, it is reasonable to assume that such pigmentary traits are under intense sexual selection and are susceptible to rapid evolutionary change.

Isozyme data (25, 26), DNA reassociation kinetics data (8), and DNA sequence data from the alcohol dehydrogenase gene (*Adh*) region (27) suggest a much closer relationship between *D. differens* and *D. planitibia* than do the mtDNA data. Hunt and colleagues (8, 28) have taken their results to indicate that the best phylogeny for these species is CI (Fig. 4). Explanations for the discrepancy between nuclear and mtDNA phylogenies presume hybridization in the history of these lineages.

The Effects of Possible Hybridization of These Species on Phylogenetic Interpretation. There are two ways to explain the discrepancy between the nuclear and mtDNA data (Fig. 5). First, the nuclear DNA phylogeny might represent the "correct" evolutionary history of these species, in which case the mtDNA data would represent evidence of hybridization. Both Ferris *et al.* (29) and Powell (30) present experimental evidence for the flow of mtDNA across a

Table 3. Wilcoxon matched-pair signed-rank tests of the maximum parsimony phylogeny (MI) vs. the phylogenies in Fig. 4

	KI	KII	KIII	SI	SII	SIII	CI	CII
− (rank sum)	0	0	0	0	0	−5	−3.5	0
Nonzero rank	14	11	15	13	10	11	9	10
Probability	****	****	***	****	***	**	*	***

For a discussion of the phylogenies in Fig. 4, see the text. ****, $P < 0.00005$; ***, $P < 0.0005$; **, $P < 0.005$; *, $P < 0.012$.

species barrier in other organisms, indicating that introgression may have a confounding effect on mtDNA phylogenies, although this is far from certain (31). Such a hypothesis would imply that the ancestor of present day *D. planitibia* hybridized with the ancestor of the two species found on the island of Hawaii (*D. silvestris* and *D. heteroneura*). The *D. silvestris–D. heteroneura* mtDNA haplotype would have to have been fixed in *D. planitibia*, with the neo-*D. silvestris–D. heteroneura* females donating the mtDNA genome. This implies that the putative *D. silvestris–D. heteroneura* donor back-migrated from Hawaii to Maui, where the hybridization event occurred, or that it existed on Maui and hybridized with *D. planitibia* before migrating to Hawaii. The Alenuihaha Channel, which separates the two islands, makes the back-migration scenario seem unlikely in the time available.

A more parsimonious alternative to explain the nuclear and mtDNA discordance is that the mtDNA phylogeny represents the more accurate evolutionary history of these species, and the nuclear DNA phylogeny is indicative of a lack of differentiation of nuclear genetic components of *D. differens* and *D. planitibia*. Founder events and associated phenomena have been suggested as a major shaping force in speciation in this group. Such founder events imply the establishment of new populations by small numbers of flies—in the extreme case, perhaps a single gravid female. Evolution via founder effects suggest that a rapidly evolving maternal marker such as mtDNA might be more sensitive in tracing subtle stochastic changes associated with founder events. Furthermore, Birky *et al.* (32) have shown that, on the average, there is a reduction by a factor of 4 in the amount of gene flow of a maternal haploid marker with respect to diploid, nuclear markers. Such a reduction of gene flow would create a situation where the nuclear components of the genomes of two populations or species could be homogenized, while at the same time a substantial amount of differentiation of mtDNA could occur. In addition, any male-mediated bias in gene flow can accentuate this sensitivity to gene flow, as has been pointed out by several authors (30, 32, 33). Mating asymmetries have been shown to exist for *D. differens* and *D. planitibia* (10) and would establish a male-mediated bias in gene flow. In particular, the more ancestral *D. differens* males would mate more successfully with the more derived *D. planitibia* females than would *D. planitibia* males with *D. differens* females, according to the asymmetry observations of Kaneshiro (10, 34). In this scenario, hybridization would have occurred on Maui Nui, probably during the Pleistocene epoch. In this epoch sea level fluctuations more than once caused the islands of the Maui complex to be joined by land bridges that were covered with continuous, lowland mesic forest (8, 35) that could well have facilitated gene flow between these populations.

Furthermore, Avise and coworkers (36, 37) predict that stochastic branching processes in organisms with well-differentiated mtDNA lineages will generate a situation where mtDNA differentiation is great, while little or no differentiation of nuclear genomes is observed (case I of fig. 8 in ref. 36). Such a case would be applicable if we accept the hypothesis of recent nuclear gene flow between *D. planitibia* and *D. differens*.

With respect to the origin of *D. silvestris* and *D. heteroneura* on the island of Hawaii, hybridization coupled with the subsequent extinction of one of the ancestral mtDNA haplotypes would confound the tracing of a double founder event, which is suggested by the morphological data (phylogenies KI and KIII). Because hybrids between *D. silvestris* and *D. heteroneura* have been captured in the field (38), the possibility of two founder events followed by hybridization deserves mention. The demonstration of ancient hybridization events is presently not possible. We emphasize, however, that if certain aspects of the population biology (i.e., founder effects and mating asymmetries) and biogeography (likelihood of gene flow over geographic barriers) are considered, both types of molecular data can reveal certain aspects of the history of these species of Hawaiian *Drosophila*. In the present case, the discordance of mtDNA and nuclear DNA phylogenies suggests that genetic contact and hybridization between the ancestors of these species may have played a significant role in the shaping of both nuclear and mitochondrial genomes.

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