ON THE ETHOLOGICAL DIFFERENTIATION OF *DROSOPHILA ANANASSAE* AND *DROSOPHILA PALLIDOSA* IN SAMOA

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A number of significant studies of genetic divergence and speciation in island populations of *Drosophila* have been undertaken in recent years. Certainly the grandest example of this has been the detailed study of the spectacular array of *Drosophila* species native to the Hawaiian Islands begun under the leadership of the late Professor Wilson S. Stone of the University of Texas and Professor D. Elmo Hardy of the University of Hawaii, and still being carried on by Hardy and a number of their colleagues; e.g., see Carson et al. (1970). Other island groups and *Drosophila* species have also been investigated by Stone and others at the University of Texas. Among these studies are those on *Drosophila ananassae* populations from island groups in the central and western Pacific Ocean, including light (brownish-yellow) and dark (blackish-brown) *D. ananassae*-like populations found on the American Samoan island of Tutuila. These populations are especially interesting in that, aside from the pigmentation difference, they are morphologically nearly indistinguishable and, in spite of exhibiting no apparent postmating reproductive barriers (Stone et al., 1966), they coexist together on this island as separate, reproductively isolated units. That they are sexually isolated has been shown cytologically (Futch, 1966) and ethologically (Futch, 1966; Spieth, 1966) with strains from Pago Pago, Tutuila.

In recent years other collections of similar light and dark *D. ananassae*-like flies have been obtained from other islands in the Samoan archipelago as well as from islands in the Fijian group. Based mainly on the cytological and ethological evidence cited above concerning the genetic isolation of these light and dark forms, Bock and Wheeler (1972) recognize the two as distinct species belonging to the *D. ananassae* subgroup of the *D. melanogaster* species group of *Drosophila*. The dark flies have been identified as *Drosophila ananassae* Doleschall, a polytypic, widespread (circumtropical) "domestic" species of the tropical and sub-tropical world, whereas they describe the light flies as *Drosophila pallidosa*. This recently described species has a relatively localized distribution presently known only to include the south-central Pacific islands of Samoa and Fiji where it exists side-by-side with its widely distributed sibling, *D. ananassae*.

That two such very closely related, potentially interfertile sympatric species have been found living together successfully and separately in the island groups of Samoa and Fiji seems to warrant further investigation. As a part of this continuing investigation, populations from other islands of the Samoan group have been analyzed cytologically and for crossing fertility as stocks have become available to me.

MATERIALS AND METHODS

The flies utilized in this study were from stocks maintained in this laboratory which were derived from collections of *D. pallidosa* and *D. ananassae* made by the late Professor W. S. Stone and Professor C. P. Oliver in each of the three major islands of the Samoan group in July–August 1965. Both species were found in collections from the vicinity of Taputimu, Tutuila, American Samoa. Both were also captured together on the island of Upolu, Western Samoa, at the Nafanua Farm near Apia. Only *D. pallidosa* was taken in the vicinity

of Aopo, Savaii, Western Samoa. The map presented in Figure 1 gives the relative positions and sizes of the islands as well as the collection sites on each of them. Electrophoretic analysis of each of the five samples showed that the two species differ significantly and consistently in Samoa in the relative frequencies of a certain set of esterase alleles, i.e., Esterase-C (Johnson et al., 1966).

An analysis of larval salivary gland chromosomes was made using the lacto-aceto-orcein technique described earlier (Futch, 1966). Between ten and twenty female larvae from each of the five stocks were examined. In order to be certain about the identification of all structural differences, hybrid larvae produced by crossing members of each stock to a stock from Majuro Atoll in the Marshall Islands, known to be homozygous for the standard *D. ananassae* banding sequence, were examined.

Metaphase chromosome configurations from larval neuroblasts were examined using the procedure described earlier (Futch, 1966).

The mating tests were done by pair-mating adult virgin flies individually in small shell vials of cornmeal, agar, sugar, and yeast medium. Although other methods for determining the extent of sexual isolation are undoubtedly more sensitive, e.g., Ehrman (1965), the no-choice method used here did prove to be adequate, provided certain precautions were taken; ether, age, temperature, and duration of contact have all been found to be very important in these kinds of experiments. All flies were collected within twelve hours of eclosion and the sexes separated while very lightly etherized. After five days, to allow for sexual maturation, the pairs were mated using an aspirator to accomplish their recovery and transfer. All matings were maintained at 26C for seven days. Mating success was determined by later examining the culture vials for the presence of at least four or more adult offspring. Since a small amount of parthenogenetic reproductive ability has been found in a few females from all of these stocks (Futch, 1972), progenies of fewer than four individuals were examined to determine if any were males. If there were no males, the cross was judged not fertile, since no individual virgin parthenogenetic female from any of these stocks has ever been observed to produce more than three offspring, and over 99% of all of these parthenogenetically produced offspring have been females. In one set of experiments, females from sterile vials were also examined for evidence of inseminations.

Chromosomal Differences

Examination of mitotic chromosomes from dividing larval neuroblasts from each of the five stocks utilized in this study confirmed the earlier observation that both species possess the same metaphase karyotype of four pairs of metacentric chromosomes: two pairs of large metacentric autosomes, a pair of small, largely heterochromatic metacentric autosomes, and a pair of medium size metacentric X chromosomes (Futch, 1966). Males have one metacentric X chromosome and a submetacentric Y chromosome. This is the basic karyotype of *D. ananassae* as described earlier by Kaufmann (1937) and Kikkawa (1938).

The results of the finer cytological analysis possible with the larval salivary gland chromosomes are shown in Table 1. In these preparations, the pair of small autosomes and the Y chromosome are included in the rather large heterochromatic aggregation forming the chromocenter and cannot be analyzed. The clear differences found earlier in the collections from Pago Pago were repeated in the later collections and therefore would presumably be found in any collection of either species from any part of Samoa. The symbols used to designate structural variations have been described and used elsewhere (Futch, 1966).

TABLE 1. *Salivary gland chromosome analysis of* D. ananassae *and* D. pallidosa *populations in the three major Samoan Islands.*

Island	Locality and Year of Collection	Chromosome	*pallidosa*	*ananassae*
Tutuila	Pago Pago (1962)	X	XLA/XLA	+/+*
		II	(2LC;2LD)/+ 2LB/+ (2RA;2RB)/+	+/+
		III	3RB/3RB	+/+
	Taputimu (1965)	X	XLA/XLA	+/+
		II	(2LC;2LD)/+ 2LB/+ (2RA;2RB)/+	2LA/+
		III	3RB/3RB	3RA/+
Upolu	Nafanua (Apia) (1965)	X	XLA/XLA	+/+
		II	(2LC;2LD)/+ 2LB/+ (2RA;2RB)/+	+/+
		III	3RB/+	+/+
Savaii	Aopo (1965)	X	XLA/XLA	None present in this collection
		II	(2LC;2LD)/+ 2LB/+ (2RA;2RB)/+	
		III	3RB/+	

* The "+" symbol indicates that the banding sequence is the same as the standard sequence of *D. ananassae*. Other symbols are explained in the text.

All of these variants in chromosome structure are paracentric inversions.

Flies from the dark populations are definitely *D. ananassae*. The connection between the Samoan dark *D. ananassae* and the body of the species is demonstrated by the discovery of two of the three inversions found in many other populations of *D. ananassae*, particularly those living in areas of large and active human habitation. Both inversion 2LA in the left arm of Chromosome II and inversion 3RA in the right arm of Chromosome III, which have been called CIIL and CIIIR in the earlier literature (Kikkawa, 1938), were found in larvae from the Taputimu *D. ananassae* stock. Other than these two rearrangements, no other chromosomal differences have been noted with respect to Seecof's (in Stone et al., 1957) standard band sequence for the salivary gland chromosomes of *D. ananassae*.

The very distinctive differentiations in chromosome structure of the light flies were found in all of the *D. pallidosa* stocks analyzed here as they were in the original study. The inverted sequence in the left arm of the X chromosome, XLA, exists in all of the collections of light flies, and, moreover, this X chromosome would seem to be never, or at least only very rarely, variable. All of these stocks are homozygous for this arrangement.

The polymorphic nature of Chromosome II originally seen in the Pago Pago collection appears to be a general phenomenon in the Samoan *D. pallidosa* populations. All of the eight possible forms have been observed. The rearrangements themselves provide some intriguing information regard-

TABLE 2. *Intraspecific crossing relationships of Samoan populations of* D. ananassae *and* D. pallidosa.

Nature of Cross ♀ ♂	Cross ♀	♂	No. Pairs	No. Fertile	% Fertile
pallidosa × *pallidosa*	T-p	× T-p	51	39	76.5
	U-p	× U-p	50	38	76.0
	S-p	× S-p	50	39	78.0
	T-p	× U-p	50	41	82.0
	T-p	× S-p	50	35	70.0
	U-p	× T-p	50	40	80.0
	U-p	× S-p	50	36	72.0
	S-p	× T-p	50	43	86.0
	S-p	× U-p	75	68	90.7
	Σ(p	× p)	476	379	79.62
ananassae × *ananassae*	T-a	× T-a	49	32	65.3
	U-a	× U-a	50	41	82.0
	T-a	× U-a	50	42	84.0
	U-a	× T-a	50	43	86.0
	Σ(a	× a)	199	158	79.39

Symbols for populations: T-p: Taputimu *pallidosa*.
T-a: Taputimu *ananassae*.
U-p: Upolu *pallidosa*.
U-a: Upolu *ananassae*.
S-p: Savaii *pallidosa*.

ing the evolutionary relationship of these two species. The small, medial inversion 2LB in the left arm of the *D. pallidosa* Chromosome II has been found in *D. ananassae* collected from Micronesia and Formosa as well as in samples of the differentiated populations of New Guinea (Futch, 1966). One member of each of the two pairs of overlapping inversions, 2LC of the (2LC;2LD) complex and 2RA of the (2RA;2RB) complex, has also been found in the collections from Micronesia and New Guinea. The evolutionary significance of overlapping inversions has been discussed by Dobzhansky (1944, 1951, 1970, and elsewhere) and has been applied by myself earlier to determine the structure of a second chromosome intermediate between these unique *D. pallidosa* sequences and the standard *D. ananassae* sequence. The evidence seems to point to the western Pacific and/or southeastern Asia as the sometime home of intermediate populations.

The variability in the right arm of Chromosome III in the collections of *D. pallidosa* from Upolu and Savaii is a departure from what had been seen before in samples from Tutuila. Inversion 3RB is common to all of the Samoan *D. pallidosa* populations. However, the standard *D. ananassae* third chromosome sequence was also found in the stocks from the two western Samoan islands. This suggests interbreeding resulting in the introduction of the standard *D. ananassae* chromosome into the *D. pallidosa* populations.

Mating Tests.—The no-choice, pair-mating tests outlined in Tables 2 and 3 demonstrated clearly that the extent of sexual isolation previously seen between Pago Pago strains of *D. ananassae* and *D. pallidosa* also exists between the two species in other parts of Samoa. The data in Table 2 indicate that intraspecific crosses showed about the same levels of success in terms of fertility, the average being about 80% for each species. However, when the crosses were interspecific, fertility dropped off sharply to 4.33% (*D. ananassae* females × *D. pallidosa* males) and 12.0% (*D. pallidosa* females × *D. ananassae* males) as Table 3 shows. The data in Table 3 generally indicate that fertile inter-

specific crosses occurred more frequently when *D. pallidosa* females were mated to *D. ananassae* males than when the reciprocal test was done. This difference is significant when the Mann-Whitney rank test is applied to the data; $T = 25.5$ ($T_{0.05} = 26$ when $n_1 = 6$ and $n_2 = 6$) and $p < 0.05$.

The data in Table 4 were obtained by pair-mating females and males in the same manner and for the same length of time as the other crosses. In these experiments, however, the spermathecae and seminal receptacles of females which could not be immediately scored as fertile were dissected out into Ringer's physiological saline and examined microscopically for the presence of sperm. In this manner, matings which would otherwise have been overlooked were detected and scored as inseminations. It is impossible to know from these experiments whether the absence of larvae from culture vials yielding such females was due to sterility on the part of one of the flies or due to very late occurring copulations, since most of the vials were inadvertently discarded prematurely. An interesting feature seen in many of these females was that the stored sperm were often quite inactive in the saline solution, something not seen in fertile females where the sperm could be seen to be very active. The reason, or reasons, for this lack of activity on the part of sperm taken from sterile females is not known. In any event, inseminated but apparently infertile females were found in nearly all types of crosses in these experiments.

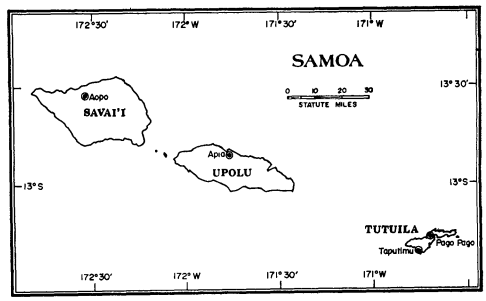

FIG. 1. The three major Samoan islands showing localities where collections were made.

The purpose of these particular matings was to examine the nature of the assortative mating behavior expected from interspecific hybrids. The results were in part anticipated in view of earlier observations made on Pago Pago hybrids (Futch, 1966). In these earlier experiments, however, the crosses were not handled in such a way as to allow reasonable comparisons of the crossing behavior of hybrids to the crossing behaviors of the parent strains. In the present set of crosses, all of the flies were handled in the same way, and the relative

TABLE 3. *Interspecific crossing relationships of Samoan populations of* D. ananassae *and* D. pallidosa.

Nature of Cross		Cross				
♀	♂	♀		♂	No. Pairs	No. Fertile	% Fertile
ananassae × *pallidosa*		T-a	×	T-p	50	1	2.0
		T-a	×	U-p	50	3	6.0
		T-a	×	S-p	50	4	8.0
		U-a	×	T-p	50	2	4.0
		U-a	×	U-p	50	1	2.0
		U-a	×	S-p	50	2	4.0
		Σ(a	×	p)	300	13	4.33
pallidosa × *ananassae*		T-p	×	T-a	50	2	4.0
		T-p	×	U-a	50	3	6.0
		U-p	×	T-a	50	8	16.0
		U-p	×	U-a	50	5	10.0
		S-p	×	T-a	50	5	10.0
		S-p	×	U-a	50	13	26.0
		Σ(p	×	a)	300	36	12.00

TABLE 4. *Crossing relationships of F_1 and Backcross (BC) Taputimu* D. ananassae-D. pallidosa *Hybrids.*

Cross	No. Pairs	No. Fertile	No. others Inseminated	No. Mated	% Mated
1. p ♀ × p ♂	97	78	7	85	87.6
2. a ♀ × a ♂	99	78	12	90	90.9
3. p ♀ × a ♂	90	7	8	15	16.7
4. a ♀ × p ♂	93	3	3	6	6.4
5. p ♀ × F_1(p ♀ × a ♂) ♂	96	76	10	86	89.6
6. p ♀ × F_1(a ♀ × p ♂) ♂	98	64	6	70	71.4
7. a ♀ × F_1(p ♀ × a ♂) ♂	100	46	13	59	59.0
8. a ♀ × F_1(a ♀ × p ♂) ♂	100	64	10	74	74.0
9. F_1(p ♀ × a ♂) ♀ × p ♂	99	80	9	89	89.9
10. F_1(a ♀ × p ♂) ♀ × p ♂	98	83	4	87	88.8
11. F_1(p ♀ × a ♂) ♀ × a ♂	100	79	3	82	82.0
12. F_1(a ♀ × p ♂) ♀ × a ♂	100	76	8	84	84.0
13. p ♀ × BC [p ♀ × F_1(p ♀ × a ♂) ♂] ♂	124	108	6	114	91.9
14. p ♀ × BC [p ♀ × F_1(a ♀ × p ♂) ♂] ♂	111	90	7	97	87.4
15. p ♀ × BC [a ♀ × F_1(p ♀ × a ♂) ♂] ♂	112	31	11	42	37.5
16. p ♀ × BC [a ♀ × F_1(a ♀ × p ♂) ♂] ♂	110	28	12	40	36.3
17. a ♀ × BC [p ♀ × F_1(p ♀ × a ♂) ♂] ♂	106	11	3	14	13.2
18. a ♀ × BC [p ♀ × F_1(a ♀ × p ♂) ♂] ♂	106	18	6	24	22.6
19. a ♀ × BC [a ♀ × F_1(p ♀ × a ♂) ♂] ♂	111	92	3	95	85.6
20. a ♀ × BC [a ♀ × F_1(a ♀ × p ♂) ♂] ♂	110	82	8	90	81.8
21. BC [p ♀ × F_1(p ♀ × a ♂) ♂] ♀ × p ♂	101	95	0	95	94.0
22. BC [p ♀ × F_1(a ♀ × p ♂) ♂] ♀ × p ♂	110	105	1	106	96.3
23. BC [a ♀ × F_1(p ♀ × a ♂) ♂] ♀ × p ♂	99	43	0	43	43.4
24. BC [a ♀ × F_1(a ♀ × p ♂) ♂] ♀ × p ♂	102	50	0	50	49.0
25. BC [p ♀ × F_1(p ♀ × a ♂) ♂] ♀ × a ♂	110	31	5	36	32.7
26. BC [p ♀ × F_1(a ♀ × p ♂) ♂] ♀ × a ♂	110	40	12	52	47.2
27. BC [a ♀ × F_1(p ♀ × a ♂) ♂] ♀ × a ♂	108	100	1	101	93.5
28. BC [a ♀ × F_1(a ♀ × p ♂) ♂] ♀ × a ♂	111	102	3	105	94.6
Σ all × p ♀ ♀	838	482	67	549	65.5*
Σ all × a ♀ ♀	825	394	58	452	54.8*
Σ all × p ♂ ♂	799	537	24	561	70.2**
Σ all × a ♂ ♂	828	513	52	565	68.2**

* Difference between mating success of p ♀ ♀ and a ♀ ♀ is significant: 2 × 2 chi-square = 19.96; $p < 0.001$.

** No significant difference between p ♂ ♂ and a ♂ ♂. 2 × 2 chi-square = 0.745; $p = 0.388$.

mating measurements should be comparable. The hybrids used in all crosses were obtained from mass matings of the appropriate parent types in half-pint milk bottles containing the same kind of culture medium as the mating vials. The choice of the two Taputimu stocks was strictly arbitrary. Backcross hybrids were obtained from crosses involving F_1 hybrid males only since it would be possible to know the X chromosome genotype precisely and to expect that little, if any, crossingover between homologs would have occurred in the heterozygous F_1. One cannot be absolutely certain about the non-existence of recombinant chromosomes in *D. ananassae* sperm since some strains of this species have been found in which genetic crossingover occurs in the spermatocytes (Kikkawa, 1937; Moriwaki, 1937; Mukherjee, 1961; Kale, 1968; Hinton, 1970). Meiotic crossingover in males is unknown in any other species of *Drosophila*. No information is yet available regarding *D. pallidosa* and crossingover in males.

Since each type of cross involved at least one of the following: *D. ananassae* females, *D. pallidosa* females, *D. ananassae* males,

or *D. pallidosa* males, it was possible to make comparisons (2 × 2 Chi-square contingency tests) of the relative mating success of *D. ananassae* females vs *D. pallidosa* females and of *D. ananassae* males vs *D. pallidosa* males—with a variety of genotypes. These data are presented at the bottom of Table 4. The results of these comparisons show that while there was a clear difference between the females, there was none between the males. These results plus those given in Table 3 show that the Samoan *D. ananassae* females must possess a greater facility for mate discrimination than the Samoan *D. pallidosa* females. Whether the males play any role in mate selection other than through their own "attractiveness" could not be determined from these experiments.

From the results of the crosses and backcrosses listed in Table 4, patterns of assortative mating exhibited by a variety of hybrid genotypes were established. The detection of these differential patterns led to the attempt which follows to analyze the differences in *D. pallidosa* and *D. ananassae* mating behavior in genetic terms. This analysis is based on the findings of Stone et al. (1966), that F_1 and F_2 interspecific hybrids of *D. pallidosa* and *D. ananassae* strains from Pago Pago are no less viable than non-hybrid progeny of these strains. The important assumption is made, therefore, that the kinds and proportions of progeny produced by any particular cross will approximate the Mendelian prediction for that cross. Since it includes only a relatively small number of genes, the small, largely heterochromatic Chromosome IV was omitted from this analysis.

Hybrid males with chromosome complements of 50% or more *D. pallidosa* autosomes and a *D. pallidosa* X chromosome crossed to *D. pallidosa* females in these experiments as well as *D. pallidosa* males did (Fig. 2). If the X chromosome was from *D. ananassae*, a small reduction in mating success was seen. Assuming that it was the X chromosome and not some type

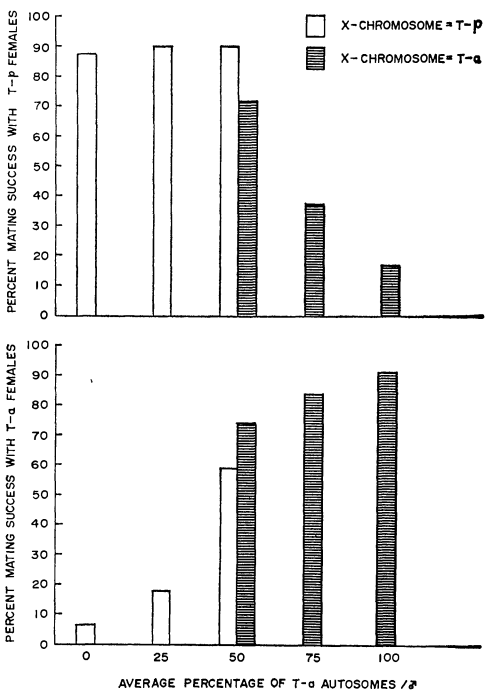

FIG. 2. Crossing relationships of the different male genotypes.

of maternal cytoplasmic inheritance that produced this difference, some genes associated with the mating behavior of *D. pallidosa* males would seem to be X-linked.

Hybrid male crosses to *D. ananassae* females showed the same type of X chromosome effect. In these crosses there appeared to be a slight reduction in mating success when the proportion of *D. ananassae* autosomes was less than 100% down to 50%. This probably reflects the greater ability of *D. ananassae* females to discriminate in mate selection.

In all of these crosses in which the proportion of alien autosomes was 50% or less, the autosomal gene loci were either homozygous for alleles from the species the hybrids were being backcrossed to or they were heterozygous. Whether the X chromosome was alien or not had an effect. In those backcrosses in which the X chromosome was alien and the proportion of alien autosomes averaged more than 50%, rela-

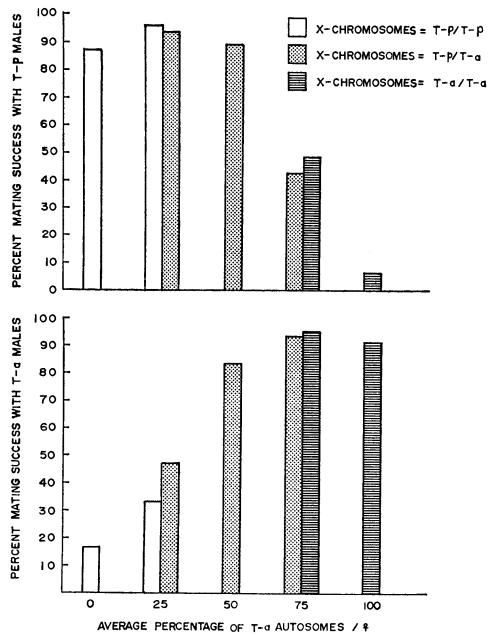

FIG. 3. Crossing relationships of the different female genotypes.

tive mating successes dropped off sharply. Many of these males, while heterozygous at some loci, were homozygous for alleles from the other species only. These observations would seem to indicate that the genes of the *D. ananassae* male mating phenotype and those of the *D. pallidosa* male mating phenotype are fairly well distributed among each of the chromosome pairs and that a heterozygote may either show parts of each phenotype separately or else an intermediate phenotype with respect to components of courtship. In any event, F_1 hybrid males were reasonably acceptable to either *D. pallidosa* or *D. ananassae* females.

Results obtained from mating hybrid females were very similar to those obtained with hybrid males (Fig. 3). In these crosses, females with complements of no more than 50% alien chromosomes, none being homozygous, backcrossed just about as well to *D. ananassae* males or *D. pallidosa* males as did *D. ananassae* females to *D. ananassae* males and *D. pallidosa* females to *D. pallidosa* males. When more than 50% of the chromosomes were alien, however, the mating frequency was reduced. These observations suggest that females with completely heterozygous complements could be courted successfully by either type of male, while, if part of the complement for mate recognition was homozygous for genes from one of the two species, some component of the ability to discriminate against males of the other species was expressed. This would seem to indicate that a female with a full haploid set of genes for each of the two female mating phenotypes could show either phenotype, responding positively to the courting stimuli provided by either kind of male most of the time.

The genes concerned with these two female mating phenotypes appeared to be well distributed among each of the two major autosome pairs. The role of X chromosome genes was not very clear. The results of crosses No. 23 (heterozygous X's) and No. 24 (homozygous *D. ananassae* X's) in which the males were *D. pallidosa* were not very different. In crosses 25 (homozygous X's) an 26 (heterozygous *D. pallidosa* X's) in which the males were *D. ananassae*, the X chromosome genotype did seem to make a difference as the former cross showed a lower degree of mating success. This is in a sense an odd result since it was the *D. ananassae* females which generally showed the greater ability for discrimination. The hybrid females in crosses 23 and 24 had 50% or more *D. ananassae* chromosomes, and the females in crosses 25 and 26 had 50% or more *D. pallidosa* chromosomes. One would expect that part of the inheritance of this greater discrimination would have been X-linked if part of the inheritance of the lesser discrimination was. It is possible of course that the males could also discriminate at this level and that the *D. ananassae* males were able to detect the difference between homozygous *D. pallidosa* X chromosome phenotype in cross 25 and the heterozygous X chromosome phenotype in cross 26.

Discussion

The larval salivary gland chromosomes and mating tests reported in this paper indicate clearly that the light and dark *Drosophila ananassae*-like populations in the Samoan Island Group are genetically differentiated, sexually isolated populations of *Drosophila*; that they are distinct species, the light being *D. pallidosa* and the dark being *D. ananassae*, as Bock and Wheeler (1972) have reported. The strongest suggestion so far of any genetic contact between them has come from the discovery that occasional females (1%–10%) from all of the Samoan *D. pallidosa* and *D. ananassae* stocks utilized in this study are capable of parthenogenetic reproduction (Futch, 1972). The only other *D. ananassae* strain known to have females with this ability is one from the islands of Tonga, 300 miles to the south of Samoa. Since the basis of this trait appears to be genetic (an asexually reproducing, all female *D. pallidosa* line from Taputimu has been successfully selected and is being maintained in this laboratory), the occurrence of such an unusual inherited characteristic in both species strongly suggests some gene flow between them.

Another line of evidence for the possibility of occasional crossing comes from the discovery here that the "standard" *D. ananassae* Chromosome III exists in the *D. pallidosa* populations on Upolu and Savaii in addition to the *D. pallidosa* Chromosome III. These two populations are polymorphic for the two gene sequences, while Tutuila *D. pallidosa* populations apparently are generally monomorphic for the *D. pallidosa* Chromosome III.

If crosses between *D. pallidosa* and *D. ananassae* occur infrequently as seems probable, the more likely of the two possible crosses would seem to be between *D. pallidosa* females and *D. ananassae* males. This conclusion is based on the laboratory tests described in this paper as well as those reported by Spieth (1966) indicating that *D. ananassae* females are less likely to hybridize than are *D. pallidosa* females. If crosses do occur, the hybrid flies would probably have almost no choice but to backcross to one of the two parental species. Only the occurrence of substantial numbers of hybrids simultaneously would present much of an opportunity for crossing between hybrids. Repeated hybridizing would be expected ultimately to lead to a blending of the two species into one unless restrictions in the form of lowered fitness of the hybrids were present. Laboratory derived evidence to date indicates no significant reduction of fitness among hybrids (Stone et al., 1966, and Futch, unpubl.), and yet the two species have remained separate and distinct. Thus, it seems reasonable to assume that, in the rare instances of hybridization that may have occurred, backcrosses followed by more backcrosses have brought about dilution of the hybrids and a slight amount of gene flow between the two species in the process of reabsorption.

Another reasonable assumption at this time is that the sharp sexual isolation separating the two species evolved during an extended period of time while their populations were geographically allopatric. The isolating mechanisms keeping them separated must have predated their present coexistence in the Samoan Islands.

The genetic complexity noted here in the inheritance of these clearly different isolating factors indicate a reasonable amount of elapsed time for their accumulation. The striking differences in the wing displays of courting *D. pallidosa* and *D. ananassae* males have been reported by Spieth (1966). Males from Pago Pago *D. ananassae* strains, as well as males from most other populations of *D. ananassae* examined, were described as characteristically spreading both wings laterally about 5° to 7° from the normal resting position and vibrating them up and down very rapidly. Male *D. pallidosa* from Pago Pago, on the other hand, were described as extending the wing closest to the female's head laterally from 50° to 90° and vibrat-

ing this wing vertically while the other wing remained in place in the resting position. During the course of the present study, numerous opportunities for observing male courtship behavior occurred when virgin flies had just been mated. All Samoan *D. ananassae* males displayed the pattern described by Spieth for Pago Pago *D. ananassae* males. Likewise, *D. pallidosa* males from all three stocks used here showed the *Drosophila melanogaster*-like wing display of the Pago Pago *D. pallidosa* males. The wing movements of F_1 hybrid males were somewhat intermediate, combining to a degree parts of the displays of both parent species. Courting hybrid males almost always extended and vibrated one wing just as *D. pallidosa* males except that the lateral angle, 20° to 40°, was somewhat smaller. On occasion, however, these same males were observed to extend and vibrate both wings like *D. ananassae*. No visual differences could be detected to differentiate F_1 hybrid males with *D. pallidosa* X chromosomes from F_1 hybrid males with *D. ananassae* X chromosomes.

Variations in the wing displays of courting *Drosophila* males may provide visual, acoustical, and/or mechanical stimuli to the females (Bastock, 1956; Manning, 1959; Ewing and Bennet-Clark, 1968; Ewing, 1969). In the case of the sibling pair of species discussed in this paper, the highly divergent nature of the wing displays produced by courting males of each species and the strong sexual isolation separating them indicate a probable importance of wing usage to them in mate recognition.

These, as well as other so far undetected differences in mating stimuli produced by males of the two species, are certainly polygenic. The complementary female receptors and, if they exist, stimuli are also polygenic. Results from backcrossing F_1 and backcross hybrids indicate this. Similar indications have been obtained by Tan (1946) from *D. pseudoobscura–D. persimilis* hybrids and by Ehrman (1961) from *D. paulistorum* semispecies hybrids. However, the traditional view of polygenic inheritance with respect to a particular trait may not adequately explain the observations reported in this study. The individual effects of certain kinds of genes governing quantitative traits may be interrelated phenotypically and yet be independent in terms of gene action (Thoday, 1967). Moreover, the stimulator and sensory phenotypes may be several and independent in terms of kinds of stimulation (acoustical, visual, olfactory, tactile, etc.), emitter organ (wing, cuticle, genitalia, etc.), and receptor organ (eye, antenna, bristles, genitalia, etc.). In the case of molecular stimuli and their receptors, it even seems possible that certain alleles could be codominant. Thus heterozygotes might show intermediate phenotypes for some mating characteristics and parts of both phenotypes for others. This latter possibility might explain in part the observed lack of discrimination where *D. ananassae–D. pallidosa* hybrids were involved.

The salivary gland chromosomes provide most of the evidence that gene flow between these two species is extremely restricted; the differences in mating behavior indicate the means by which this restriction is imposed. The successful coexistence of the two species may be due to differences in habitat selection. As I have noted from observations made in the field on Tutuila (Futch, 1966), *D. pallidosa* is not restricted to areas affected by human activities such as villages, cultivated crop plants, etc., although it is found there, whereas *D. ananassae* is probably a more recent colonizer and is concentrated in and around towns and villages. In addition, Narise (1966) has supplied experimentally obtained information concerning the migratory behavior of the two which suggests that they would tend to respond to one another so that they would not always occupy exactly the same habitats. This apparent difference in habitat selection, plus the strength of their sexual differences may help to explain the successful sympatry of this pair of potentially interfertile sibling species in the confines of these islands.

Summary

The extent of the sexual isolation separating populations of a sympatric, sibling pair of species, *Drosophila ananassae* Doleschall and *Drosophila pallidosa* Bock and Wheeler, from the three major islands of Samoa has been examined cytologically and ethologically. The evidence obtained indicates that the two species are genetically distinct and that their separation is maintained mainly by strong mating discrimination. Some indication has been found, however, that occasional crosses may have occurred between the two species in nature. Nevertheless, these incidents must have been rare and the fertile hybrids they produced were probably reabsorbed by the parent populations.

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