

Incipient reproductive isolation between *Drosophila nasuta* and *Drosophila albomicans*

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Summary – Genetic divergence was investigated between 2 closely related allopatric species, *Drosophila nasuta* and *D albomicans*, which have not evolved sexual (pre-mating) isolation. Several post-zygotic components of fitness were analysed in intra- and inter-specific hybrids. The results show that these 2 species are reproductively isolated because they are different co-adapted systems. Among geographic populations of *D albomicans*, genetic differentiation has also occurred. The evidence for primary subspeciation in *D albomicans* is discussed.

Drosophila nasuta subgroup / fitness component / speciation / subspeciation

Résumé – Début d'isolement reproducteur entre *Drosophila nasuta* et *Drosophila albomicans* – On a analysé la divergence génétique entre 2 espèces affines allopatriques, *Drosophila nasuta* et *D albomicans*, qui n'ont pas développé d'isolement sexuel précopulatoire. Plusieurs composantes post-zygotiques de la fitness ont été analysées chez les hybrides intraspécifique et interspécifiques. Les résultats montrent que les 2 espèces sont isolées au point de vue reproduction parce-qu'elles ont des systèmes coadaptés différents. Parmi les populations géographiques de *D albomicans*, une différenciation génétique s'est aussi produite. La possibilité d'une subséciation primaires chez *D albomicans* est discutée.

sous-groupe *Drosophila nasuta* / composantes de la fitness / spéciation / subséciation

INTRODUCTION

The *Drosophila nasuta* subgroup of the *immigrans* species group consists of more than 10 species and subspecies, therefore, this subgroup is well suited for the study of the genetics of speciation. Members of the *D nasuta* subgroup are widely distributed from the Pacific Ocean to Africa, through South-east Asia and the Indian Oceans areas (Kitagawa *et al*, 1982). This subgroup is comparable to the *willistoni* species group as subjects for the study of speciation mechanisms. However, a conspicuous difference exists between these 2 groups: the former are island

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populations, whereas the latter are continental. This distinction may produce a fundamental difference in their mode of speciation.

For the study of the speciation mechanisms, analyses of genetic differentiation at the first step of speciation processes among closely related taxa should be instructive. *D nasuta* and *D albomicans* are very closely related species. Sajjan and Krishnamurthy (1972) and Rajasekarasetty *et al* (1979) regard *D albomicans* as a subspecies of *D nasuta*. However, there is a striking difference in karyotypes between the two. The basic karyotype of this group is $1V + 2R + 1D$ (ie, $2n = 8$), yet from among all members of this group, only *D albomicans* has $2V + 1D$ ($2n = 6$), a result of fusions of the third and the sex chromosomes (Wakahama and Kitagawa, 1972; Wakahama *et al*, 1983).

D nasuta had been collected in the Seychelles, Madagascar, Mauritius, Reunion, India and the east coast of Africa, mainly by one of the authors (Kitagawa). David and Tsacas (1981) reported *D nasuta* on the west coast of Africa, and they assumed that the expansion in the distribution of this species occurred by a form of man-made transportation.

D albomicans is distributed from south-western parts of Japan and Taiwan to the south-east of the Asian continent, Burma, Cambodia, China, India, Malaysia and Thailand. The "dot" chromosome of *D albomicans* (No. 4 for the subgroup) differs depending on locality. The Japanese and Taiwanese populations have the longer type of "dot" chromosomes, which consists of large heterochromatic regions. The other populations have shorter "dot" chromosomes (Wilson *et al*, 1969; Wakahama *et al*, 1983; Hatsumi, 1987).

D nasuta and *D albomicans* cannot be distinguished by morphology. No sexual reproductive isolation has been detected between these 2 species, and both male and female F_1 inter-specific hybrids are fertile (Kitagawa *et al*, 1982), but in some crosses the F_1 hybrids males are almost sterile (Hatsumi and Kitagawa, unpublished data). The most common alleles at the *Est- α* and *Est- β* isozyme loci are the same for both species, but frequencies of alleles differ, thus indicating that genetic differentiation has occurred between the 2 species (Kitagawa *et al*, 1982). The karyotypes of fertile hybrids of *D nasuta* and *D albomicans* (as *D n nasuta* and *D n albomicans*, respectively) have been studied over many generations by Ramachandra and Ranganath (1986). They have demonstrated that, in certain cases, stable "Cytoraces" evolve, wherein individuals possess chromosomes originating from both species. We have shown (Inoue and Kitagawa, 1974; 1975) that the fitness of hybrids varies greatly depending on the origin of the founding strains; the frequency of sterility in F_1 hybrid males ranges from 3.2 to 4.8%.

The present study further clarifies the genetic differentiation between *D nasuta* and *D albomicans*; we investigated certain components of fitness relevant to post-mating reproductive isolation and show that hybrid breakdown occurs in subsequent generations following interspecific hybridization. Furthermore, we discuss the apparent primary subspeciation among local populations of *D albomicans*.

MATERIALS AND METHODS

Six geographical strains were used: Okinawa, Japan (designated as OKA), Wulai, Taiwan (FOR), and Chiangmai, Thailand (CNX) for *D albomicans*; Kandy, Sri

Lanka (KDY), Mahé, Seychelles (SEZ), and Mombasa, Kenya (MBA) for *D nasuta* (Fig 1). Each geographic strain was established by pooling iso-female lines, which descended from single wild-caught mated females. Ten iso-female lines were used for OKA and FOR, 5 lines for CNX, 4 lines for KDY, 10 lines for SEZ and 6 lines for MBA. After 2 generations following pooling of the cultures, the experiments were started.

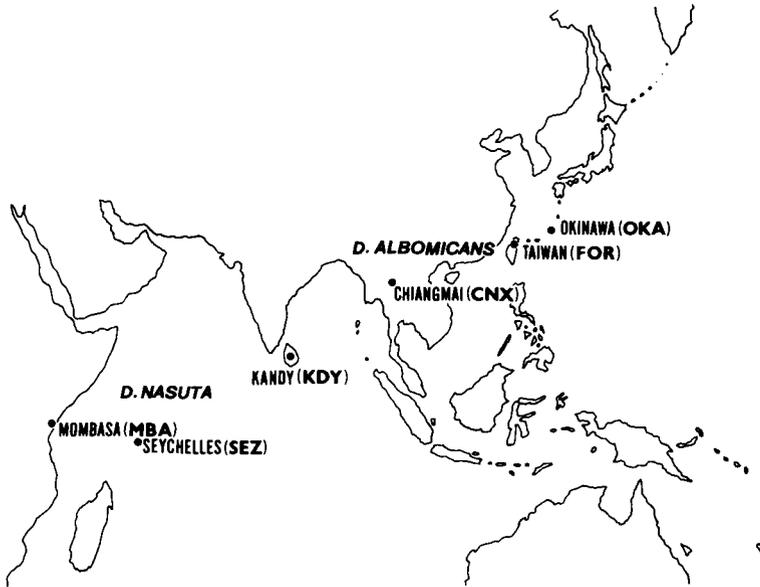


Fig 1. Collection sites of *D nasuta* and *D albomicans* strains used in this experiment.

All possible diallel crosses were simultaneously undertaken with the 6 strains. For each cross, 15 females and 20 males were put into a vial immediately after eclosion, and were kept together for 7 days to ensure mating. Their progeny (F_1) were obtained from the vial, and thereafter, the F_2 and F_3 flies were reared by successive sib-matings. In all experiments, except the productivity experiment, 4 replications were set up for each cross in the successive 3 generations.

Insemination rate

Following the 7-day mating period, females were dissected to examine spermathecae and seminal receptacles for the presence of sperm. The proportion of the fraction (inseminated females/total females) is regarded as the insemination rate.

Pre-adult viability

On the next day of the mating period, 50 eggs were sampled from each cross and placed in a new vial containing the standard cornmeal-molasses medium. The number of flies which emerged from each vial was counted until the 16th day after

the eggs had been sampled. The proportion of the number of adult flies to eggs is regarded as the pre-adult viability.

Hatchability

After the 7-days mating period, flies were transferred to a new vial containing partly modified Delcour's (1969) medium for 10 h. Two days later, the number of hatched and unhatched eggs were counted. The proportion hatched/total eggs is regarded as hatchability.

Productivity

After the mating period, flies were transferred everyday to a new vial containing 20% boiled yeast medium, for 8 days. The number of progeny from the 1st, 3rd, 5th and 7th cultures, and the number of eggs laid on the 2nd, 4th, 6th and 8th days were counted to determine productivity.

Sex ratio

The number of male and female progeny ($\sigma^7 / (\sigma^7 + \text{♀})$), produced on standard medium, were recorded from samples of all crosses over the 3 generations. All experiments were carried out at $25 \pm 1^\circ\text{C}$.

RESULTS

Insemination rate

A total of 6 480 females were dissected to examine sexual organs for the presence of sperm. The results are shown in Table I. All the intra-population crosses showed high insemination rates, except for CNX, which showed only a 50% insemination. In the intra-specific crosses of *D nasuta*, most values did not differ significantly from the parental values, except that the F_2 generation of MBA ♀ × SEZ ♂ showed a significantly higher rate, and the F_1 of MBA ♀ showed a lower rate than the mid-parent value, comparing confidence limits about means. In 17 combinations out of 54 inter-specific generation combinations, significantly lower insemination rates were observed, especially in the F_1 and F_3 generations.

Hatchability

The results are shown in Table II. In total, 135 949 eggs were counted to estimate the hatchability, from which 112 969 eggs hatched. The average hatchability in the parental strains was 91.8%. In the intra-*albomicans* crosses, 3 cases of hybrid breakdown were detected among 18 crosses. In the intra-*nasuta* crosses, all 6 2nd-generation crosses had higher values than the parental strains, 3 of which were significant. No hybrid breakdown appeared in any generation.

In the crosses of *albomicans* ♀ × *nasuta* ♂, no hybrid vigour was observed and the number of breakdown crosses increased with the generations: 2 in the 1st, 6 in the 2nd, and 9 (all) in the 3rd-generation crosses had lower hatchability. In

Table I. Insemination rate (%) in the original strains and their hybrids at F_1 , F_2 and F_3 generations.

♀	♂	<i>D albomicans</i>			<i>D nasuta</i>		
		<i>OKA</i>	<i>FOR</i>	<i>CNX</i>	<i>KDY</i>	<i>SEZ</i>	<i>MBA</i>
O	F_1		100.0	93.3	98.3	98.0	98.3
K	F_2	99.4	100.0	98.3	98.3	100.0	96.5
A	F_3	±0.6	98.3	98.3	72.5 ⁻⁻⁻	78.8 ⁻⁻⁻	89.5
F	F_1	100.0		43.0	73.0 ⁻⁻⁻	96.8	89.8
O	F_2	95.0 ⁻	98.3	90.0	100.0	100.0	95.0
R	F_3	98.3	±1.2	81.5	88.0 ⁻⁻⁻	95.0	96.5
C	F_1	70.5	68.3		76.5	100.0	75.8
N	F_2	26.0 ⁻⁻⁻	49.0	50.0	76.5	64.3	66.3
X	F_3	52.0	49.8	±5.8	31.5 ⁻⁻⁻	63.5	68.5
K	F_1	51.8 ⁻⁻⁻	80.0 ⁻	96.5		100.0	100.0
D	F_2	80.0	88.0 ⁻	50.0	98.8	95.0	96.5
Y	F_3	37.8 ⁻⁻⁻	49.3 ⁻⁻⁻	78.0	±0.7	96.5	98.3
S	F_1	88.3	94.8	67.5	96.5		94.8
E	F_2	91.5	96.8	80.0	100.0	97.8	96.8
Z	F_3	89.8	88.3 ⁻	88.3	98.3	±1.2	95.0
M	F_1	57.8 ⁻⁻⁻	79.0 ⁻	50.0	62.5 ⁻⁻⁻	91.5	
B	F_2	79.3 ⁻⁻⁻	98.3	35.3	96.5	98.3 ⁺	94.2
A	F_3	27.5 ⁻⁻⁻	54.8 ⁻⁻⁻	25.8 ⁻⁻⁻	86.0	87.3	±3.4

Three generations were pooled in the original strains. Marks of + and - show hybrid vigour and hybrid breakdown, respectively; 3 marks (---) mean significance at the 0.001, at the 2 (--) 0.01 and 1 (-) at the 0.05 levels, applying confidence limits about means.

the crosses of *nasuta* ♀ × *albomicans* ♂, 2 out of 9 crosses in the F_1 generation showed hybrid breakdown, 3 out of 9 in the F_2 generation, and all 9 in the F_3 generation showed significant hybrid breakdown. Thus, the results for hatchability were approximately the same in the reciprocal crosses.

Pre-adult viability

In total, 21 600 eggs were sampled from which 11 618 flies emerged (Table III and Fig 2). In contrast to the hatchability, the original strains apparently carried different mutations affecting larval mortality. The average viability was only 68.6% in the original strains. The value of the CNX strain was considerably lower (34.7%).

From the intra-*albomicans* and intra-*nasuta* crosses, significant vigour appeared in the intra-*nasuta* 2nd- and 3rd-generation crosses (8 crosses out of 12). Among

Table II. Egg hatchability (%) in the original strains and their hybrids (other explanation are the same as those of Table I).

♂		<i>D albomicans</i>			<i>D nasuta</i>		
♀		<i>OKA</i>	<i>FOR</i>	<i>CNX</i>	<i>KDY</i>	<i>SEZ</i>	<i>MBA</i>
O	<i>F</i> ₁		89.3	92.9	90.1 ⁻⁻	91.7	87.3 ⁻⁻⁻
K	<i>F</i> ₂	95.3	95.5	93.5	53.5 ⁻⁻⁻⁻	31.5 ⁻⁻⁻⁻	58.3 ⁻⁻⁻⁻
A	<i>F</i> ₃	±0.6	93.1	74.3 ⁻	61.9 ⁻⁻⁻⁻	60.1 ⁻⁻⁻⁻	59.3 ⁻⁻⁻⁻
F	<i>F</i> ₁	94.8		85.9	93.0	94.9	92.1
O	<i>F</i> ₂	81.1 ⁻⁻	92.1	97.1	90.3	84.4	92.3
R	<i>F</i> ₃	90.8	±1.3	93.3	81.6 ⁻⁻	74.4 ⁻⁻	78.2 ⁻⁻⁻⁻
C	<i>F</i> ₁	91.6	84.6		89.4	82.0	86.2
N	<i>F</i> ₂	53.9 ⁻⁻⁻⁻	86.5	87.8	44.6 ⁻⁻⁻⁻	42.3 ⁻⁻⁻⁻	48.0 ⁻⁻⁻⁻
X	<i>F</i> ₃	81.1	83.3	±2.0	64.8 ⁻⁻⁻⁻	74.1 ⁻⁻⁻⁻	73.7 ⁻⁻
K	<i>F</i> ₁	85.1	89.3	78.6		90.5	94.6
D	<i>F</i> ₂	91.2 ⁻	95.2	37.5 ⁻⁻⁻⁻	94.5	98.3 ⁺⁺	95.6
Y	<i>F</i> ₃	39.2 ⁻⁻⁻⁻	50.9 ⁻⁻⁻⁻	67.9 ⁻⁻⁻⁻	±0.6	92.8	92.0
S	<i>F</i> ₁	84.5	86.6	63.4 ⁻⁻⁻⁻	92.1		89.7
E	<i>F</i> ₂	88.4	84.3	84.5	96.3	90.5	96.0
Z	<i>F</i> ₃	66.4 ⁻⁻⁻⁻	70.1 ⁻⁻⁻⁻	54.8 ⁻⁻⁻⁻	92.9	±1.8	93.2
M	<i>F</i> ₁	90.1	83.5	77.9 ⁻	89.8	89.3	
B	<i>F</i> ₂	89.4	91.2	59.3 ⁻⁻⁻⁻	98.8 ⁺⁺	98.3 ⁺	90.8
A	<i>F</i> ₃	76.7 ⁻⁻⁻⁻	65.8 ⁻⁻⁻⁻	58.4 ⁻⁻⁻⁻	88.7	94.9	±1.5

the inter-specific crosses, significant breakdown appeared in the 2nd- and 3rd-generations of *albomicans* ♀ × *nasuta* ♂ (8 and 6 crosses out of 9, respectively). In crosses of *nasuta* ♀ × *albomicans* ♂, hybrid breakdown was observed mainly in the *F*₁ and *F*₃ generations. Only in the *F*₂ generation of *KDY* ♀ × *FOR* ♂ was hybrid vigour detected.

Productivity

The patterns of daily productivity of eggs and progeny were similar to the parental strains. When males and females mated just after emergence, fertile eggs were not laid for a few days and the peak egg-laying capacity was between 7 and 10 days after emergence. In total, 167 437 eggs and 80 126 progeny were counted; the results are shown in Tables IV and V. On average, a female of the original strain laid 20.4 eggs per day, from which 12.6 flies emerged.

For egg productivity in the intra-*albomicans* crosses, both hybrid vigour and breakdown appeared in every generation. In the intra-*nasuta* crosses, 5 crosses

Table III. Pre-adult viability (%) of the original strains and their hybrids (other explanations are the same as those of Table I).

♀	♂	<i>D albomicans</i>			<i>D nasuta</i>		
		OKA	FOR	CNX	KDY	SEZ	MBA
O	<i>F</i> ₁		80.0	62.5	73.5	66.5	66.0
K	<i>F</i> ₂	79.2	87.0 ⁺	65.0	15.5 ⁻⁻⁻⁻	26.0 ⁻⁻⁻⁻	21.5 ⁻⁻⁻⁻
A	<i>F</i> ₃	±2.2	85.0	70.5	47.5 ⁻⁻⁻⁻	60.5	49.0 ⁻⁻⁻⁻
F	<i>F</i> ₁	40.5 ⁻⁻⁻⁻		64.5	46.5 ⁻⁻⁻⁻	62.0	51.3 ⁻⁻⁻⁻
O	<i>F</i> ₂	68.0	68.5	45.0	62.5	25.5 ⁻⁻⁻⁻	58.0 ⁻⁻⁻
R	<i>F</i> ₃	88.5 ⁺	±3.1	54.0	55.0	53.0 ⁻⁻⁻⁻	52.5 ⁻⁻⁻⁻
C	<i>F</i> ₁	19.0	49.5		60.5	46.5	69.0
N	<i>F</i> ₂	2.0 ⁻⁻⁻⁻	12.5 ⁻⁻⁻⁻	34.7	20.5 ⁻⁻⁻	20.0 ⁻⁻⁻⁻	16.5 ⁻⁻⁻⁻
X	<i>F</i> ₃	43.0	39.0	±2.5	23.0 ⁻⁻⁻	39.5	20.5 ⁻⁻⁻⁻
K	<i>F</i> ₁	49.5 ⁻⁻⁻	72.5	62.0		73.0	68.5
D	<i>F</i> ₂	81.0	81.0 ⁺⁺⁺	20.5 ⁻⁻⁻	59.5	70.5	53.0
Y	<i>F</i> ₃	26.5 ⁻⁻⁻⁻	32.5 ⁻⁻⁻⁻	19.0 ⁻⁻⁻⁻	±2.4	60.0	78.5 ⁺⁺
S	<i>F</i> ₁	43.5 ⁻⁻⁻⁻	48.5 ⁻⁻⁻⁻	48.0	55.5		72.5
E	<i>F</i> ₂	72.5	59.5	47.5	79.0 ⁺⁺	66.8	80.5 ⁺⁺⁺
Z	<i>F</i> ₃	35.0 ⁻⁻⁻⁻	21.0 ⁻⁻⁻⁻	26.0 ⁻⁻⁻	78.5 ⁺⁺	±2.2	79.5 ⁺⁺⁺
M	<i>F</i> ₁	42.5 ⁻⁻⁻⁻	37.0 ⁻⁻⁻⁻	53.0	38.0 ⁻⁻⁻⁻	62.0	
B	<i>F</i> ₂	61.0	72.5	63.5	78.5 ⁺⁺	91.0 ⁺⁺⁺	69.0
A	<i>F</i> ₃	42.0 ⁻⁻⁻⁻	15.0 ⁻⁻⁻⁻	32.0	82.0 ⁺⁺⁺	68.0	±1.5

out of the 6 second-generation crosses showed hybrid vigour, averaging 29.4 eggs/female/day.

In the crosses of *albomicans* ♀ × *nasuta* ♂, hybrid vigour appeared more frequently than breakdown in the 1st and 2nd generation, but hybrid breakdown was frequently shown in the 3rd generation. In the crosses of *nasuta* ♀ × *albomicans* ♂, hybrid breakdown appeared in 5 crosses out of the 9 third-generation crosses. In productivity, hybrid breakdown was clear in the inter-specific 3rd generation of both sets of reciprocal crosses.

The number of progeny produced in the intra-*albomicans* crosses indicate vigour in 3 cases of the 1st- and 3rd-generation crosses each, and hybrid breakdown occurred in 3 second-generation crosses and in 1 of the third-generation crosses. In the intra-*nasuta* crosses, all 6 crosses showed hybrid vigour in the 2nd generation. In the crosses of *albomicans* ♀ × *nasuta* ♂, hybrid vigour appeared in the 1st generation (6 cases out of 9), and hybrid breakdown was seen in the 2nd (8 out of 9) and the 3rd generations (6 out of 9). In the crosses of *nasuta* ♀ × *albomicans* ♂,

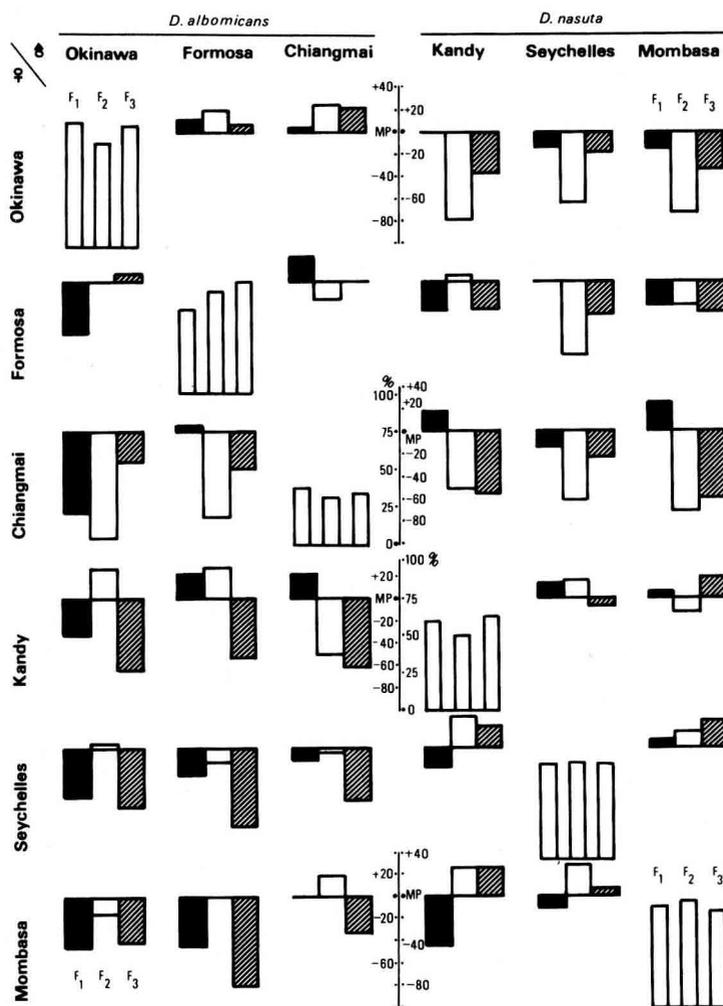


Fig 2. Pre-adult viability (%) of the original strains and their hybrids in 3 successive generations.

all 9 cases showed significant hybrid breakdown in the 3rd generation. In general, the results of progeny productivity were similar to those of egg fecundity.

Sex ratio

The sex ratio (numbers of males/total flies) of the original strains and their hybrids in the 3 successive generations are shown in Fig 3. Altogether, 69 981 females and 66 306 males were counted. In each replication vial, more than 100 flies were

Table IV. The number of eggs per female per day in the original strains and their hybrids. Marks of + and - mean significant hybrid vigour and hybrid breakdown, respectively.

♀	♂	<i>D albomicans</i>			<i>D nasuta</i>		
		OKA	FOR	CNX	KDY	SEZ	MBA
O	F ₁		17.6 ⁻	19.4 ⁺	20.7 ⁺	18.1 ⁻	14.8 ⁻
K	F ₂	18.8	16.7 ⁻	21.7 ⁺	17.5	20.0	25.0
A	F ₃		17.5 ⁻	23.5 ⁺	9.4 ⁻	21.0	19.6
F	F ₁	23.5 ⁺		24.5 ⁺	23.1 ⁺	24.9 ⁺	24.2
O	F ₂	18.1 ⁻	21.6	26.4 ⁺	26.1 ⁺	21.2 ⁺	25.7 ⁺
R	F ₃	20.8		19.3	22.6 ⁺	21.9	21.3 ⁻
C	F ₁	17.6 ⁻	20.9		18.3	28.7 ⁺	26.0 ⁺
N	F ₂	23.0 ⁺	15.1 ⁻	19.1	17.8	23.2 ⁺	27.7 ⁺
X	F ₃	18.9	17.1 ⁻		17.1	15.8 ⁻	16.6 ⁻
K	F ₁	27.7 ⁺	24.1 ⁺	14.9 ⁻		16.3	13.2 ⁻
D	F ₂	16.6	24.0 ⁺	17.1	15.3	24.6 ⁺	24.3
Y	F ₃	16.8	12.3 ⁻	11.4 ⁻		20.4	19.8
S	F ₁	21.9	21.2 ⁻	22.5	25.9 ⁺		17.7 ⁻
E	F ₂	19.9	21.5 ⁻	18.8 ⁻	31.0 ⁺	22.7	39.0 ⁺
Z	F ₃	18.5 ⁻	17.1 ⁻	22.0	21.2		25.2 ⁻
M	F ₁	22.6	20.4 ⁻	21.9	25.9 ⁺	30.2 ⁺	
B	F ₂	36.2 ⁺	32.3 ⁺	28.1 ⁺	27.0 ⁺	30.6 ⁺	25.1
A	F ₃	20.3	21.6	16.0 ⁻	21.6	24.9	

counted. No differences were found among the 6 original strains, *ie*, $48.9 \pm 0.9\%$ on average.

In the intra-*albomicans* crosses, 3 cases out of 18 showed significant distortion; the 2nd generation of OKA ♀ × FOR ♂ showed a marked excess of males ($p < 0.01$), although the reciprocal cross was normal. Significantly fewer males ($p < 0.05$) were observed in the F₃ generation of FOR ♀ × OKA ♂ and the F₂ generation of FOR ♀ × CNX ♂. In the intra-*nasuta* crosses, all cases were normal.

An abnormal sex ratio was frequently detected in the inter-specific crosses. In 27 cases of *albomicans* ♀ × *nasuta* ♂ crosses, 9 showed reductions in the numbers of males (OKA ♀ × *nasuta* ♂, and FOR ♀ × *nasuta* ♂), whereas a significant excess of males was observed only in the F₂ generation of CNX ♀ × KDY ♂. In the cross of FOR ♀ × KDY ♂, significant male under-representation was observed in every generation. From crosses of OKA ♀ and FOR ♂ to all *D nasuta* males, drastic male breakdown was detected in the F₂ generation, with only 8.6% males produced by OKA ♀ × SEZ ♂; yet, these males were fertile. In 27 cases of *nasuta* ♀ × *albomicans* ♂, the situation was quite different when compared with the reciprocal

Table V. The number of progeny per female per day in the original strains and their hybrids. Marks of ⁺ and ⁻ have the same meaning as in Table IV.

♂		<i>D albomicans</i>			<i>D nasuta</i>		
♀		<i>OKA</i>	<i>FOR</i>	<i>CNX</i>	<i>KDY</i>	<i>SEZ</i>	<i>MBA</i>
O	<i>F</i> ₁		19.2 ⁺	13.0	12.6	16.2 ⁺	14.5
K	<i>F</i> ₂	13.2	14.8	11.4	7.8 ⁻	5.9 ⁻	10.6 ⁻
A	<i>F</i> ₃		18.1 ⁺	15.0 ⁺	5.3 ⁻	13.9 ⁺	9.5 ⁻
F	<i>F</i> ₁	24.4 ⁺		23.2 ⁺	17.5 ⁺	24.8 ⁺	25.0 ⁺
O	<i>F</i> ₂	11.4 ⁻	15.4	14.4	15.6 ⁺	8.0 ⁻	12.1 ⁻
R	<i>F</i> ₃	20.6 ⁺		12.3	16.8 ⁺	15.4	10.5 ⁻
C	<i>F</i> ₁	9.7	14.2		13.3 ⁺	13.5 ⁺	10.1
N	<i>F</i> ₂	1.2 ⁻	2.2 ⁻	7.3	2.3 ⁻	4.8 ⁻	2.7 ⁻
X	<i>F</i> ₃	9.6	5.2 ⁻		2.8 ⁻	6.0 ⁻	4.6 ⁻
K	<i>F</i> ₁	12.3	20.7 ⁺	7.4		7.8 ⁻	7.8 ⁻
D	<i>F</i> ₂	7.9 ⁻	14.1	6.2 ⁻	11.8	18.6 ⁺	19.0 ⁺
Y	<i>F</i> ₃	5.9 ⁻	2.6 ⁻	2.2 ⁻		11.2 ⁻	10.9 ⁻
S	<i>F</i> ₁	14.3 ⁺	12.7 ⁻	10.8	14.6 ⁺		12.1 ⁻
E	<i>F</i> ₂	13.5 ⁺	10.0	8.2	18.0 ⁺	13.3	22.5 ⁺
Z	<i>F</i> ₃	8.9 ⁻	5.4 ⁻	7.2 ⁻	18.5 ⁺		18.3 ⁺
M	<i>F</i> ₁	12.5 ⁻	19.5 ⁺	22.8 ⁺	17.8 ⁺	22.2 ⁺	
B	<i>F</i> ₂	19.3 ⁺	17.8 ⁺	7.8	18.5 ⁺	18.4 ⁺	14.7
A	<i>F</i> ₃	6.2	3.7 ⁻	1.3 ⁻	15.5 ⁺	14.6	

crosses. Six cases of male excess and only 6 cases of male reduction were seen. In contrast, the 2nd generation of the inter-specific crosses was characterized by a large excess of females from *albomicans* ♀ × *nasuta* ♂, and by a male excess from the reciprocal *nasuta* ♀ × *albomicans* ♂.

DISCUSSION

The genetic constitutions of natural populations of diploid organisms are integrated, co-adapted gene complexes being produced by evolutionary processes (Dobzhansky, 1970). Hybrids between local populations which have evolved different genetic systems frequently show hybrid vigour. When genetic differences between 2 populations are large, integrated genetic systems can be broken following hybridization. Co-adapted linkage association can be disrupted by recombination between chromosomes derived from separate localities and ultimately destroyed in subsequent generations. The phenomenon called "hybrid breakdown" has been documented

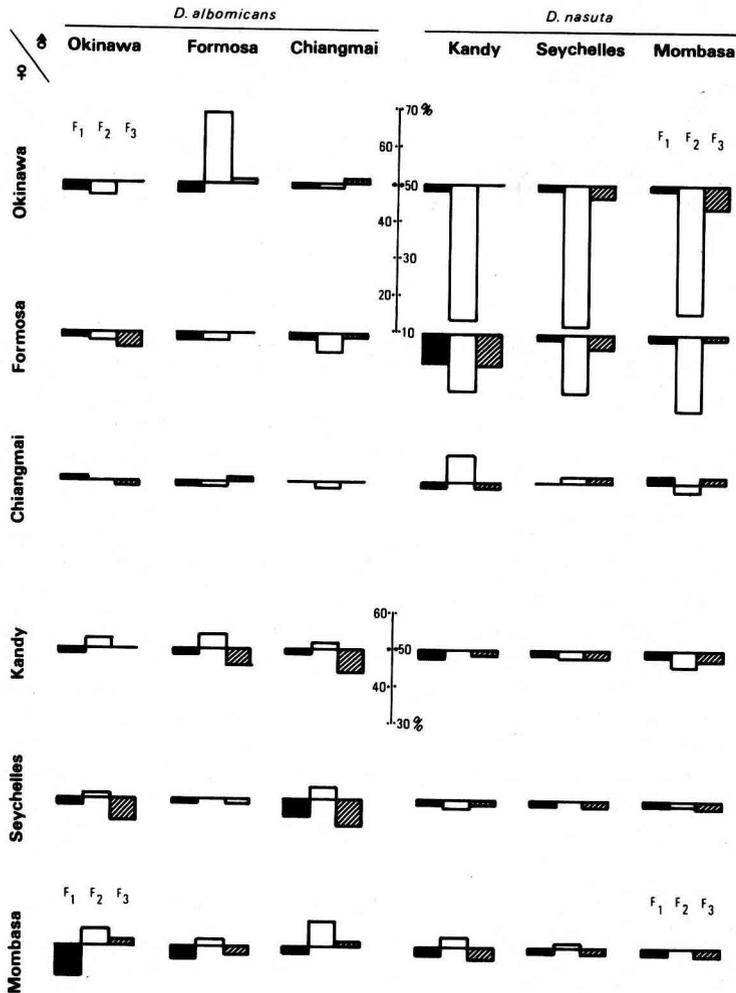


Fig 3. The sex-ratio (numbers of males/total flies) of the original strains and their hybrids in 3 successive generations.

in *D pseudoobscura* (Vetukhiv, 1954, 1956; Brncic, 1954, 1961; Orr, 1987) and in *D melanogaster* (Wallace, 1955).

The results of the present study demonstrate that significant genetic divergence has occurred between 2 closely related members of the *Drosophila nasuta* subgroup (*D nasuta* and *D albomicans*), as indicated by hybrid breakdown. Furthermore, inter-population crosses within species show that greater genetic divergence exist among *D albomicans* populations than among local populations of *D nasuta*.

Intra-population crosses

These served as control experiments for both intra- and inter-specific crosses. All strains except the Chiangmai (CNX) showed high fitness in the various characteristics examined. For the CNX, the insemination rate was about half, and pre-adult viability and the number of progeny were less than half, compared to the other 5 strains. However, the number of eggs laid and egg hatchability were normal. The low fitness of the CNX population might partly be attributed to the existence of supernumerary chromosomes in this population (Hatsumi and Kitagawa, 1980; Hatsumi, 1987).

Inter-strains within species crosses

This is one of the useful scales to determine differentiation within species. In *D albomicans*, the F_2 breakdown was commonly seen in combinations of crosses measuring the number of progeny and pre-adult viability. Remarkable F_2 breakdowns were observed from crosses between CNX females to OKA and FOR males. In addition to the existence of the supernumerary chromosomes mentioned above, CNX have shorter "dot" chromosomes compared to those of OKA and FOR populations. The results of pre-adult viability indicated that the CNX population has genetically differentiated from the island populations, OKA and FOR. Subspeciation among geographic populations of *D albomicans* is discussed below.

For *D nasuta*, the number of progeny from crosses of KDY ♀ × SEZ ♂ MBA ♂ was lower in the F_1 and F_3 generations and higher in the F_2 generation. The F_1 hybrids of MBA ♀ × KDY ♂ showed extremely low pre-adult viability (38%). Yet, in many cases, hybrids between local populations showed hybrid vigour. This seems to suggest that geographic populations of *D nasuta* have sufficiently differentiated to exhibit heterosis (hybrid vigour). However, compared to *D albomicans*, the genetic differentiation among local populations of *D nasuta* is less advanced. This is consistent with David and Tsacas's (1981) suggestion that *D nasuta* has only recently expanded its distribution to the Indian Ocean area and to Africa.

Inter-specific crosses

The insemination rates of inter-specific hybrids in the F_1 and F_2 generations were significantly lower than those of the parents, with lower rates in 10 out of 18 combination-generation crosses of *D nasuta* ♀ and *D albomicans* ♂. The reciprocal crosses, in which KDY or MBA were used as the male parents, showed insemination rates in the F_1 and F_3 generations.

As to egg hatchability, 4, 9 and 18 crosses out of 18 showed lower values than did their lower parent in the F_1 , F_2 and F_3 generations, respectively (Table II). In about half the numbers of combinations in the F_1 and F_2 generations, the numbers of progeny produced by a female per day were significantly higher than in the parental strains. F_1 heterosis was seen in 10 out of 18 crosses, but hybrid breakdown was frequently demonstrated in the subsequent F_2 and F_3 generations (Table V). In all crosses of *D nasuta* ♀ × *D albimocans* ♂ in the F_3 generation, the numbers of progeny decreased significantly. These results support the conclusion that *D nasuta* and *D albomicans* have different co-adapted genetic systems. The hybrid breakdown

in the F_3 generation is partly attributed to the major karyotype difference between the 2 species (Sato *et al.*, 1977).

Hybrid breakdown in pre-adult viability was detected in the F_2 and F_3 generations of the crosses of *D albomicans* ♀ × *D nasuta* ♂ and also observed in the F_1 and F_3 generations in the reciprocal crosses. In the former case, the F_1 flies had $2n = 7$, and abnormal segregation of chromosomes in meiosis of the F_1 males should be the main source of genetic imbalance (Sato *et al.*, 1877).

The striking sex-ratio distortion in the F_2 generation of the cross between *D albomicans* ♀ × *D nasuta* ♂ was of great interest. In other crosses, we did not find any significant distortion in the F_1 , F_2 and F_3 generations, except for the F_2 generation of OKA ♀ × FOR ♂ in *D albomicans*. However, in the F_2 generation of the crosses between *D albomicans* ♀ × *D nasuta* ♂ drastic decreases of males were observed. The average recovery of males in the F_2 generation was 11.2% in OKA ♀ × *D nasuta* ♂, and 32.2% in FOR ♀ × *D nasuta* ♂.

Two fitness components, egg hatchability and pre-adult viability, assess developmental processes of hybrids, and thus, the components of fitness examined in this study demonstrate that the reduction in fitness observed among hybrid recombinants can be attributed to the differentiation of co-adapted genetic systems. It is evident that significant differences in genetic constitution between *D nasuta* and *D albomicans* have evolved since hybrids have reduced fitness at the post-zygotic stage.

As a by-product of genetic differentiation, *nasuta* and *albomicans* produce hybrids with reduced fitness. Since the genetic divergence has arisen in allopatry, it cannot be said to be caused by hybridization; post-zygotic reproductive isolation of this type conforms with the first phase of Dobzhansky's (1951) speciation model; hybridization and reinforcement being the second phase (see also Ayala *et al.*, 1974). We believe that *nasuta* and *albomicans* are incipient species at the first phase of a 2-phase speciation process.

Differentiation among *D albomicans* strains

Hybrid breakdown was commonly observed in egg hatchability and in pre-adult viability (egg to adult). The hybrids between more genetically remote strains are expected to die at earlier developmental stages. The value "1 - (pre-adult viability)" can be regarded as total mortality, and "1 - (egg hatchability)" as embryonic mortality. If the ratio of (embryonic mortality)/(total mortality) is large, it means a large fraction of hybrids die early in their development. Therefore, this ratio can be used as a measure of genetic differentiation between geographic strains. The average ratio was calculated from the data in Tables II and III.

The degree to which embryonic death contributes to pre-adult mortality of F_1 , F_2 and F_3 generations in all 36 combinations of crosses is shown in Table VI. The proportion of embryonic mortality to total mortality in inter-specific hybrids steadily increased with generations. The intra-specific crosses of *D albomicans* show a similar pattern of increasing embryonic mortality with generations, but to a lesser degree than the inter-specific crosses. This pattern suggests that geographic populations of *D albomicans* have achieved substantial genetic divergence, perhaps to the level of semispecies. The inter-specific and intra-*albomicans* pattern of embryonic mortality is not evident among *D nasuta* inter-locality crosses.

Table VI. The proportion of embryonic mortality to total mortality in various levels of crosses.

	<i>Embryonic mortality / Total mortality</i>				
	<i>No of crosses</i>	<i>Generation</i>			<i>Mean</i>
		<i>F₁</i>	<i>F₂</i>	<i>F₃</i>	
Original strain					
<i>D albomicans</i>	3	0.18	0.22	0.29	0.23
<i>D nasuta</i>	3	0.26	0.23	0.22	0.24
Intra-specific crosses					
<i>D albomicans</i>	6	0.27	0.30	0.48	0.35
<i>D nasuta</i>	6	0.27	0.13	0.33	0.24
Inter-specific crosses					
<i>albomicans</i> ♀ × <i>nasuta</i> ♂	9	0.27	0.52	0.57	0.45
<i>nasuta</i> ♀ × <i>albomicans</i> ♂	9	0.38	0.47	0.54	0.46

Moreover, significant hybrid breakdown was observed in pre-adult viability of the F_1 and F_3 generations of CNX ♀ × OKA ♂ the F_2 and F_3 generations of CNX ♀ × FOR ♂, and in the F_1 generation of FOR ♀ × OKA ♂; whereas, in the intra-*nasuta* crosses, hybrid breakdown was seen only in the F_1 generation of MBA ♀ × KDY ♂. Sex-ratio distortion of hybrids (the reduction of males) was quite different among 3 strains of *D albomicans* (OKA, FOR and CNX) in the F_2 generation of *albomicans* ♀ × *nasuta* ♂ crosses. This is further evidence of differentiation at the genic level among *albomicans* strains. The population located farthest from the distribution border of *D nasuta*, the OKA population of *D albomicans*, showed an extreme sex-ratio distortion in the F_2 generation; the next farthest population, the FOR, was the next in the degree of distortion. The CNX population, located nearest to *D nasuta*, showed a normal sex ratio (similar to intra-*nasuta* crosses). These observations support the following conclusion: the 3 populations of *D albomicans* (OKA, FOR and CNX) are undergoing primary subspeciation. However, further studies are necessary to determine the specific genetic differences between *D nasuta* and *D albomicans*, and among the geographic populations of *D albomicans*.

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