As in this study, the sensitive period for phenocopy induction is generally found to occur at an earlier stage of development than the expression of the equivalent genetic form. For example, the sensitive period for the induction of the bithorax phenocopy is only 2–2.5 h after ovipositing (the preblastoderm stage), compared with the formation of the wing imaginal disc which occurs during the 3rd instar stage[19, 20]. The results presented here illustrate that for y_p, the sensitive period precedes the major peak of both yellow mRNA expression, which occurs at the late pupal stage[7, 8 21] and the associated process, melanization, which occurs during day 4 of pupal development[8, 22]. Therefore, the sensitive period for the induction of the phenocopy occurs well before any of the known associated processes are initiated. Hence α-DMT is probably not *directly* affecting gene expression/action, or its associated developmental process, as expected by Goldschmidt[23] and Scott[14] respectively.

A possible route of induction of a yellow cuticle might be by a build up in the hemolymph of tyrosine residues which are subsequently converted to dopa[24], the main precursor of the melanization/sclerotization pathways[24, 25]. Since the degree of sclerotization is dependent on the amount of sclerotizing agent[26], phenocopy induction through the ingestion of the excess tyrosine in the form of α-DMT may be expected.

Because α-DMT induces a change in mating behavior[9, 10], as well as the cuticle of *Drosophila melanogaster*, the 3rd instar is also a sensitive period in the development of the Specific-Mate Recognition System (SMRS)[27, 28]. One possibility is that sexual behavior is being directly affected by α-DMT. Burnet and Wilson[6], for example, suggested that expression of the mutant yellow gene in the sexual foci residing in the thoracic ganglion may result in the impairment of mating ability. The chemical agent α-DMT might be affecting the sexual foci in a similar way. The sexual focus in the thoracic ganglion comprises one possible structure that interacts with α-DMT resulting in a divergent SMRS. Other possible structures, not related to the development of sexuality, that may be affected through the same third instar sensitive period, are the nervous system and the cuticle of the *Drosophila* itself[9].

If the sensitive period tends to occur prior to the expression of the associated gene or gene-complex, it seems unlikely that phenocopy induction is simply the 'suppressing, retarding or disorienting effect on one or more gene-controlling components of the normal or mutant genotype'[29]. In fact induction can occur via a number of different developmental pathways. Not only can the workings of the genetic nexus be directly affected by the inductive agent, as suggested by Blanc and Child[30] but, for example, in the case of y_p, induction may occur through increasing the amount of substrate (tyrosine) in the associated melanization and sclerotization pathways.

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Density of flies and male-crowding affect the outcome of interspecific crosses between *Drosophila simulans* and *D. mauritiana* and the hybrid progeny numbers

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Summary. When *Drosophila simulans* females from the Seychelles are crossed with *D. mauritiana* males the number of hybrids produced decreases with an increase in both density of flies and male/female proportion. The patterns are consistent in the reciprocal cross although the number of progeny is reduced. The asymmetry of mating success affects not only mating preference and the outcome of the cross, but also the progeny numbers.

Key words. Hybridization; asymmetrical mating success; male/female proportion; hybrid progeny number; *Drosophila mauritiana; Drosophila simulans.*

Table 1. Number of hybrid offspring produced in interspecific crosses between *Drosophila simulans* and *D. mauritiana* at various densities and proportions of males. Above A) ♀ *simulans* × ♂ *mauritiana;* Below B) the reciprocal cross ♀ *mauritiana* × ♂ *simulans*. n: number of parental females; tx: number of tubes tested; t: number of tubes that successfully produce hybrid progeny; T: total cumulative number of hybrid adults produced by the entire series of tubes; per ♀: idem per female per 20 days, results are corrected to unities; SR: sex ratio (♂:♀ + ♂).

		n♀×n♂					n♀×2n♂					n♀×4n♂				
	n	tx	t	T	per ♀	SR	tx	t	T	per ♀	SR	tx	t	T	per ♀	SR
A)	1	26	26	6973	268	0.48	16	16	4708	294	0.50	12	12	2652	221	0.48
	5	14	14	8523	122	0.48	12	12	4712	78	0.50	12	12	4555	76	0.49
	10	12	12	8923	74	0.49	12	12	4012	33	0.59	12	12	4313	36	0.49
	20	13	13	6868	26	0.48										
B)	1	21	1	143	7	0.51	12	4	571	48	0.50	20	4	343	17	0.37
	5	12	8	2265	38	0.46	12	10	1765	29	0.41	12	8	484	8	0.42
	10	12	8	1969	16	0.41	12	12	1274	11	0.45	12	7	724	6	0.43
	20	12	10	2661	11	0.42										

Table 2. Between-isofemale line comparison in *Drosophila mauritiana* of the number of hybrid offspring produced when crossed with *D. simulans* from the Seychelles. *mau.* line ref.: *D. mauritiana* isofemale line reference in the Gif stock (see table 1 for further legends).

mau	♀ *sim* × ♂ *mau*					♀ *mau* × ♂ *sim*				
line ref.	tx	t	T	per ♀	SR	tx	t	T	per ♀	SR
75	6	6	2187	36	0.48	10	8	1689	17	0.45
60	10	9	4195	42	0.51	10	5	907	9	0.36
76	10	10	5974	60	0.50	12	2	36	0.3	0.44
72	10	10	7027	70	0.49	10	6	1021	10	0.43
67	10	10	7407	74	0.48	11	2	414	3.8	0.46
69	10	10	8662	87	0.50	10	5	723	7	0.39
74	10	10	8893	89	0.50	12	7	980	8	0.43

Drosophila mauritiana is an insular endemic species restricted to Mauritius, while *D. simulans* is an expanding cosmopolitan species also present on the Indian Ocean islands except Mauritius[2].

Drosophila simulans and *D. mauritiana* are very closely related species and chromosomally homosequential[3–5]. Male progeny of the *D. mauritiana* × *D. simulans* cross, and its reciprocal, are sterile, whereas females are fertile. Testes develop fully but they are agametic[6,7]. Female hybrids are fertile when backcrossed to either species[7,8]. Therefore, although the two species are usually allopatric, occasional dispersal may result in genetic introgression[9]. The hybridization results are true, irrespective of the geographical origin of the *D. simulans* strain used, except for the Seychelles strain. Seychelles *D. simulans* males and *D. mauritiana* females usually fail to produce progeny together. Yet, by increasing the proportion of males, the likelihood of success increases[7]. Similarly, interspecific hybridization between *D. melanogaster* and *D. simulans* increases proportionately with an increase in the number of males[10].

Here we investigate the effects of density and male crowding on the outcome of interspecific matings between *D. mauritiana* and the Seychelles strain of *D. simulans*. We analyzed both the frequency of mating and the number of offspring produced. We also considered the variations between isofemale lines of *D. mauritiana* in giving uneven numbers of offspring in interspecific crosses with *D. simulans* from the Seychelles.

The *Drosophila simulans* strain (Gif. 229-2) used in the experiments originated from Mahé Is., Seychelles (1981) and the *D. mauritiana* strain (Gif. 163-1) is from Chaland Is., Mauritius (1973). Both were founded by more than one wild-caught female. The isofemale lines (i.e., founded from one female) of *D. mauritiana* (Gif. 275-1) were from Les Galets, Mauritius (1985). Newly eclosed males and females were confined at 21 °C in tubes containing standard cornflour medium. For 20 days they were transferred to fresh vials every five days. Three different experimental protocols were used: in the first protocol, parental females and males were in equal proportions; in the second, males were twice as numerous as females, and in the third, males were four times as numerous as females. Each of these three protocols was used for a series of tests where the quantity of flies increases as indicated on table 1. The numbers and sex of resulting offspring were recorded. Each cross was repeated at least 12 times. The variability of isofemale lines of *D. mauritiana* was estimated by crossing each with *D. simulans* from the Seychelles strain at a density of 10 ♀ + 10 ♂ under the same conditions as above, each cross being repeated 10 times (table 2).

Table 1 shows that the average number of hybrid offspring produced per female in 20 days varies greatly. A striking similarity is observed in the cross between *D. simulans* females and *D. mauritiana* males in the three protocols: a gradual decrease in the number of hybrids produced, with increasing number of flies. In the first (n ♀:n ♂) protocol there is a gradual decrease in the amount of hybrids produced per female when the number of flies increases. This decrease is 55% between 2 and 10 flies, 72% between 2 and 20, and 95% between 2 and 40. In the second (n ♀:2n ♂) protocol the same values are 73% between 2 and 10 flies, and 89% between 2 and 20; and in the third (n ♀:4n ♂) protocol, it is 66 and 84% respectively. This difference is statistically significant (Chi-square test, $p < 0.05$) between the series involving two flies and those involving 10, 20 and 40 flies respectively, whereas it is not significant between the last three. In the reciprocal cross the number of hybrids produced is exceedingly low. Nevertheless, it shows a similar decrease with both fly density and male proportion, but to a lesser extent than in the normal cross. It is unclear why the cross involving one *mauritiana* female and one *simulans* male results in such a striking reduction in the number of hybrid progeny (table 1). Hence, the asymmetry of mating success affects both the outcome of the cross and the numbers of the hybrid progeny as well as the mating preference[11]. However, the asymmetric production of hybrids is not pronounced at the highest densities.

The number of hybrid offspring decreases significantly when males outnumber females (Chi-square test, $p < 0.01$), yet there is no difference when the number of males is greater than 2n. However, there is an interesting exception with one female associated with two males, which produces the largest number of hybrid offspring in both directions of cross (i.e., 294 and 48).

Table 2 shows the between-isofemale line variability. Some *D. mauritiana* lines can produce double the number of hybrids relative to others. The between-line variability implies that the founder females differ[12]. Nevertheless, the average number of hybrid progeny is in the same order of magnitude (65 hybrids) as that yielded by the *D. mauritiana* strain (74 hybrids) at the same density (see table 1).

A strong asymmetry is consistently observed irrespective of the isofemale line tested (Chi-square test, $p < 0.01$). There is a normal sex ratio in the hybrid progeny from the cross between *D. simulans* females and *D. mauritiana* males. By contrast, there is a noticeable, although non-significant, deficiency of males (Chi-square test, $p < 0.05$) in the reciprocal cross between *D. mauritiana* females and *D. simulans* males. This is true of the results shown in both table 1 and table 2. The success of the hybridization (t) of the cross of *D. simulans* females from the Seychelles with *D. mauritiana* males confirms earlier results[7], irrespective of the density of flies and male/female proportion (tables 1 and 2). By contrast, a noticeable deviation is observed in the reciprocal cross, that is between *D. mauritiana* females and *D. simulans* males from the Seychelles. Our results are only partially consistent with a previous report[7]. We obtained hybrids in 53 % of the crosses involving *D. mauritiana* strains and 47 % of those using *D. mauritiana* isofemale lines, whereas earlier hybridization experiments completely failed to produce hybrids. Nevertheless, when successful, the cross led to a highly variable number of offspring. All the hybrid males dissected (30 per category of cross) showed normal, although aspermic, gonads but immature cysts were observed.

In our study, the success of the reciprocal cross (t) increases with an increase in the number of flies but not in the male/female proportion (table 1). Many workers have concluded that density influences mating success but for different, sometimes conflicting[13–16] reasons. Our results show that the increasing proportion of males does not affect the mating success between *D. mauritiana* and *D. simulans,* whereas it was shown to increase that between *D. melanogaster* and *D. simulans*[11]. Similarly, it was reported[16] that the mating success of *vestigial* males is enhanced by the presence of winged (*ebony*) males, suggesting that the behavior of females may be shifted by the presence of more than one male.

In the Hawaiian lek species, *D. grimshawi,* the frequency of courtship displays was linearly dependent upon male density but agonistic and communal displays were density-dependent only when females were present[17]. However, it was also argued that when males outnumbered females this factor significantly slowed mating[16]. Otherwise, if larval overcrowding could account for the discrepancy between the increasing number of parental females and hybrid offspring[18,19], it cannot explain the decrease of the progeny number with an increasing proportion of parental males.

In conclusion, strong asymmetry exists not only in the frequency of mating but also in the number of hybrid progeny. As a consequence, the results are nearly the same when one compares those obtained in the normal cross at higher densities and in the reciprocal cross at lower densities. Asymmetry is complex at different levels in the frequency of interspecific mating[11,20], in the extent of overlap in sexual signals[21,22], and in the effects of sexual selection[22]. Moreover, there is asymmetry in the numbers of hybrids produced per female which necessarily involves some kind of postzygotic factor.

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Ecdysteroid conjugates in the ovaries of the silkworm, *Bombyx mori*: 3-phosphates of 2,22-dideoxy-20-hydroxyecdysone and of bombycosterol

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Summary. Two novel ecdysteroid conjugates, 2,22-dideoxy-20-hydroxyecdysone 3-phosphate (**1**) and bombycosterol 3-phosphate (**2**), as well as four known ecdysteroid 22-phosphate esters, have been isolated and characterized from the ovaries of the silkworm, *Bombyx mori.*

Key words. Ecdysteroid conjugate; *Bombyx mori*; silkworm; 2,22-dideoxy-20-hydroxyecdysone 3-phosphate; bombycosterol 3-phosphate.