

Evolution of a lesser fitness trait: egg production in the specialist *Drosophila sechellia*

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Summary

In the evolutionary process during which *Drosophila sechellia* became specialized on a toxic fruit (morinda), a spectacular decrease in female reproductive capacity took place when compared with the species' generalist relatives *D. mauritiana* and *D. simulans*. Comparisons of species and interspecific crosses showed that two different traits were modified: number of ovarioles and rate of egg production. During the conservation of a *D. sechellia* strain on usual food, adaptation to laboratory conditions led to an increase in the rate of oogenesis but not in ovariole number. Comparison of F₁ and backcross progeny also suggests that the two traits are determined by different genes (ovariole number has already been shown to be polygenic). When morinda is available as a resource, the low rate of egg production in *D. sechellia* is partly compensated by a stimulating effect, while an inhibition occurs in *D. simulans*. It is assumed that *D. sechellia* progressively adapted itself from rotten, non-toxic morinda to a fresher and more toxic resource. During this process the rate of oogenesis evolved from an inhibition to a stimulation by morinda. Simultaneously a spectacular decrease in ovariole number took place, either as a consequence of stochastic events related to the small population size of *D. sechellia* and a metapopulation dynamics, or as an adaptive process favouring dispersal capacities of the female.

1. Introduction

Studies of ecological communities generally show that species richness is accompanied by a diversification of ecological niches (Hutchinson, 1978) and that the number of species coexisting at a trophic level is limited. In *Drosophila*, it has been argued (Shorrocks & Rosewell, 1984) that a guild of related species with overlapping niches would not exceed an average number of seven.

A usual way for avoiding strong competition is resource partitioning, i.e. evolution towards specialization (Hutchinson, 1978; Thompson, 1994). Phytophagous or parasitic groups offer numerous examples of specialized species. In *Drosophila*, many generalist species are able to use a diversity of resources, but many others are reduced to a narrow

ecological niche. A much-studied case is the *D. repleta* group, in which many species coevolved with Cactaceae (Heed & Mangan, 1986).

Evolution towards specialization is likely to occur when a given resource is both abundant and predictable. The evolutionary mechanisms and genetic changes which are necessary remain poorly investigated, however. The main reason is that specialization is generally observed between different species where complete reproductive isolation precludes genetic analyses. Among a few favourable cases on different host races or sibling species, we may mention the work of Feder *et al.* (1988) on *Rhagoletis*, of Thompson (1988) on butterflies and of R'Kha *et al.* (1991) on *D. sechellia*. The last species, which is restricted to the Seychelles archipelago, breeds only in the toxic fruit of *Morinda citrifolia*. Compared with its sibling generalist species, *D. mauritiana* and *D. simulans*, which presumably exhibit ancestral character states, *D. sechellia* is remarkable by its tolerance to morinda toxicity, by an adult attraction to this smelly fruit, and an oviposition preference by the females (R'Kha *et al.*, 1991). Such differences explain the niche divergence

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on an adaptive basis, leading to an avoidance of interspecific encounters and competition. Indeed we were able to keep the two species coexisting for more than 6 months in the same population room but on different resources – *D. simulans* on banana and *D. sechellia* on morinda (unpublished results).

Another striking difference between the two species is the adult reproductive potential, which is very low in *D. sechellia* (Louis & David, 1986; R'Kha *et al.*, 1991; Coyne *et al.*, 1991) so that laboratory cultures of *D. sechellia* are difficult to keep on the usual food. From an evolutionary point of view, we see no obvious reasons why specialization should be accompanied by a severe reduction in egg production.

In the present study we tried to achieve a better understanding of the specialization of *D. sechellia* by means of a comparison with its two generalist siblings, i.e. *D. simulans* and *D. mauritiana*. Two components of fecundity – ovariole number and rate of oogenesis – were analysed. The reactivity of the three species to various amounts of morinda was investigated and a comparison of an old and new *D. sechellia* strain presented. The evolutionary hypotheses which may explain the results are discussed.

2. Materials and methods

(i) *Drosophila* populations and hybrids

Experiments were done with two natural populations from the Seychelles, collected in 1985. For *D. simulans*, 40 isofemale lines were collected on Mahé island; the lines were then pooled into a single mass population and kept in culture bottles. *D. sechellia* was collected on Cousin islet near Praslin island. More than 100 flies, wild collected or emerging from fallen morinda fruits, were pooled to make a mass culture. The third, closely related species, *D. mauritiana*, was collected on Mauritius in 1988 (David *et al.*, 1989). Again a mass culture was established by pooling more than 30 isofemale lines. The three species were kept at 20 ± 2 °C in bottles on usual cornmeal–sugar *Drosophila* medium. At each generation, the number of parent flies was greater than 200. For *D. sechellia*, which is difficult to keep in laboratory conditions, the initial population was subdivided into three parallel strains, kept in different places. Periodic mixing of the three strains was performed in the course of the experiments. Some comparisons were also made with an older mass culture of *D. sechellia*, collected in 1981, also on Cousin islet.

Genetic investigations were made by crossing *D. simulans* and *D. sechellia*. Female *D. simulans* were mated to *D. sechellia* males since this cross is much easier than the reciprocal one (Lachaise *et al.*, 1986; R'Kha *et al.*, 1991). Hybrid females, which are normally fertile, were backcrossed to both parental species.

(ii) Egg production

Experimental flies were reared at 25 °C with a low larval density on a high-nutrient, killed-yeast medium (David & Clavel, 1965). Upon emergence, adults were manipulated by aspiration, without anesthesia, and single pairs were established in small egg-laying cages (volume 190 ml). An egg-laying plate (surface 4.5 cm²) was fitted at the bottom of each cage and changed every day. Eggs were counted daily. Oviposition took place on the usual cornmeal–sugar food, seeded with live yeast. In some experiments a weighed amount of morinda was placed on the egg-laying plate. Fruits of morinda were collected in French Polynesia and kept frozen until use.

The duration of an experiment was generally 15 days. At the end, each female was dissected and the number of ovarian tubes, or ovarioles, was counted. Maximum daily egg production was averaged over days 4–8 for each female. This value was divided by the ovariole number to obtain the rate oogenesis, i.e. the average number of eggs produced by an ovariole in a day.

3. Results

(i) Egg production on normal food: comparison of the three sibling species

Egg production curves (Fig. 1) exhibited an overall triangular shape as already known in *D. melanogaster* (David *et al.*, 1974). Oviposition begins on the second day of female life and increases linearly up to day 4. Then a slow, progressive and linear decrease occurs according to female age. A striking observation is the very low egg production of *D. sechellia*, with a maximum of about 20 eggs a day, as against 60 or 80 in *D. mauritiana* and *D. simulans* respectively. This low fecundity is the consequence of two independent

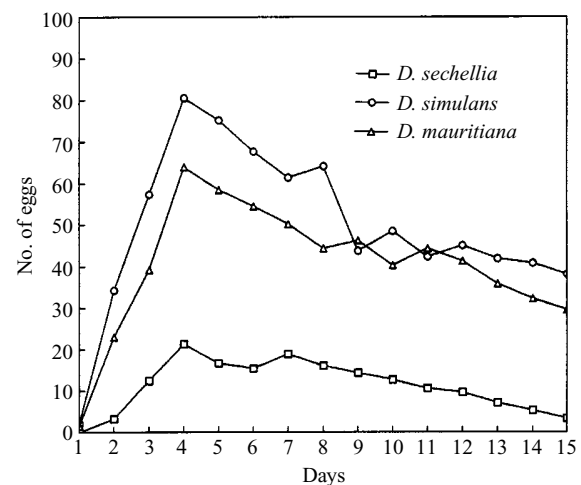


Fig. 1. Daily egg production curves on normal food in three sibling *Drosophila* species.

Table 1. Relationship between ovariole number and rate of egg production on normal food in *D. sechellia*, *D. simulans* and *D. mauritiana*

Species	n	Ovariole		Max. F	Rate	r
		number				
<i>D. sechellia</i>	67	17.7±0.3	20.5±0.4	1.2±0.02	0.14	NS
<i>D. simulans</i>	55	35.4±0.3	72.4±1.3	2.0±0.04	0.36	**
<i>D. mauritiana</i>	34	28.1±0.5	61.2±1.3	2.2±0.50	0.20	NS

Results of females on different experiments were pooled. n, number of females; ovariole number, mean number of ovarioles with standard error; Max. F, maximum daily fecundity (days 4–8); Rate, rate of egg production per ovariole per day; r, correlation coefficient between max. F and ovariole number.

** p < 0.01 (one-tailed test).

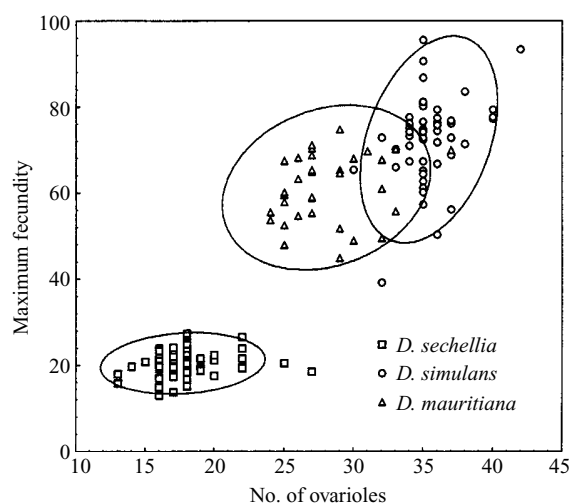


Fig. 2. Relationship between number of ovarioles of each female and maximum daily egg production. For each species, 0.95 confidence ellipses are shown.

characteristics acting in synergy (Table 1). *D. sechellia* is characterized by a very small ovariole number (about 18 vs 28 and 35 in the other species) and a reduced rate of oogenesis (1.2 egg per ovariole vs 2.0 and 2.2).

The relationship between ovariole number of each female and its maximum fecundity (Fig. 2) again contrasts the species. A broad overlap exists between the two generalist species, while *D. sechellia* has a distinctly lower rate of oogenesis. If we assume that the rate of oogenesis is stable, we should find a positive, strong correlation between ovariole number and maximum fecundity. In fact, the within-species phenotypic correlations are very low (Table 1) and significant only in *D. simulans*. Such low values are due to the fact that the rate of oogenesis is highly variable among females. In *D. sechellia* it ranged between 0.7 and 1.5 (CV 17%), while the ranges were 1.2–2.7 (CV 13%) in *D. simulans* and 1.5–2.7 (CV 15%) in *D. mauritiana*. For this trait, which describes resource allocation to reproduction, a minor overlap is found between *D. sechellia* and *D. simulans*.

(ii) Reactivity to morinda; effects of increasing amounts of morinda

Results obtained with three different amounts of morinda (70, 250 and 500 mg) per egg-laying plate are summarized in Table 2. In *D. sechellia*, stimulation of egg production was observed in each case, but no difference according to the amount of fruit. Since ovariole number was the same, the increased fecundity is due to an increased rate of egg production (R'Kha *et al.*, 1991). For the generalist species, the picture was quite different. The lowest amount (70 mg) failed to show any significant effect. Greater amounts significantly reduced egg production, i.e. exhibited a toxic

Table 2. Fecundity comparison (egg production during 10 days) of *D. sechellia*, *D. simulans* and *D. mauritiana* with different amounts of morinda and without morinda

Species	Food	Amount of morinda (mg)					
		70	n	250	n	500	n
<i>D. sechellia</i>	Control	132.4 ± 5.5	15	157.1 ± 8.9	7	157.1 ± 8.9	7
	Morinda	189.9 ± 3.4	14	212.4 ± 7.9	9	198.3 ± 9.1	9
	Difference	+57.5 ± 6.5		+55.3 ± 11.9		+41.2 ± 12.8	
	t	8.88*		4.65**		3.21**	
<i>D. simulans</i>	Control	534.9 ± 28.5	12	590.7 ± 14.2	9	602.1 ± 21.7	14
	Morinda	563.7 ± 28.6	10	506.0 ± 10.8	9	453.1 ± 16.1	9
	Difference	+28.8 ± 40.6		-84.7 ± 17.8		-149.0 ± 8.5	
	t	0.71 NS		4.75**		5.22**	
<i>D. mauritiana</i>	Control	421.9 ± 8.4	13	527.5 ± 21.1	8	521.6 ± 11.4	13
	Morinda	423.3 ± 10.2	15	440.1 ± 13.7	7	384.2 ± 31.1	6
	Difference	+1.5 ± 13.3		-87.4 ± 25.7		-137.5 ± 30.2	
	t	0.11 NS		3.40**		4.55**	

n, number of females; Difference, difference between fecundity with morinda and fecundity without morinda. t, Student's t test; ** p < 0.01.

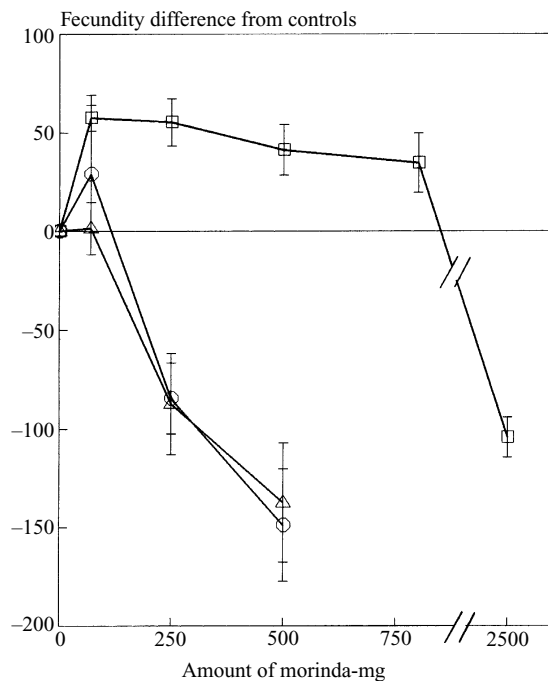


Fig. 3. Influence of increasing amounts of morinda on total egg production at 10 days in the three related species. Egg production is shown by considering the difference between controls (no morinda) and morinda-treated flies. The maximum divergence between species is observed with 500 mg of morinda. Vertical bars indicate the confidence intervals. Symbols are the same as in previous figures.

effect on oogenesis. Still higher quantities were then used, i.e. 800 and 2500 mg per plate. With 800 mg, a significant increase in egg production compared with controls was still observed in *D. sechellia*. For the two other species the toxicity was such that most females died before the end of the experiment. The largest

amount (2500 mg) did not result in a premature death of *D. sechellia* but in a significant decrease in its egg production, indicating a real toxicity for that tolerant species.

Differences between species are best evidenced by comparing the response curves in relation to the amount of morinda (Fig. 3). For the generalist species, morinda is indifferent in small quantity, but becomes rapidly toxic when the amount increases so that egg production is less than in control flies. For *D. sechellia*, morinda is mostly beneficial, and very large quantities are necessary to get a toxic effect. The maximum difference between species is observed with 500 mg morinda.

The influence of 500 mg was investigated in hybrid progeny between *D. sechellia* and *D. simulans* (R'kha et al., 1991). Since morinda increased the rate of oogenesis in *D. sechellia* and decreased that of *D. simulans*, the rates of egg production were quite similar among genotypes (Fig. 4A). On the other hand, the picture becomes much clearer when considering, for each genotype, the difference between treated and control females (Fig. 4B). We see that the toxic effect is fully expressed in the F₁, i.e. the sensitivity of *D. simulans* is dominant. No significant effect is found in backcross *D. sechellia* females.

(iii) Evolution of *D. sechellia* under laboratory conditions

Since *D. sechellia* can be grown in the laboratory, and indeed was kept without morinda on usual *Drosophila* food, we may expect some adaptation to such new laboratory conditions. All the above-described experiments were done with a mass culture established in 1985. Another mass population, collected in 1981,

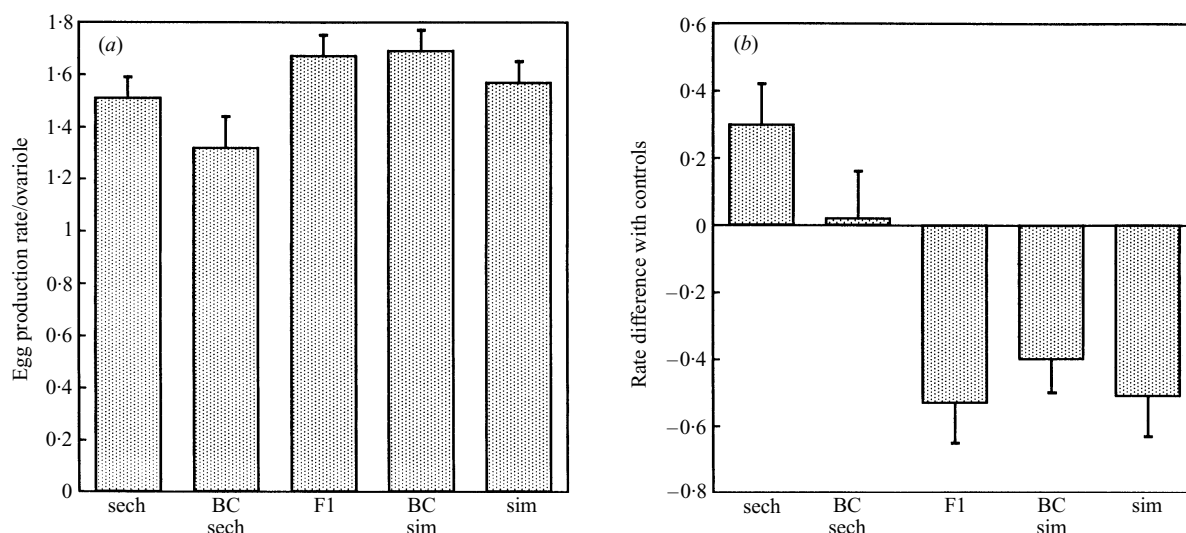


Fig. 4. Influence of morinda (500 mg) on the daily rate of egg production per ovariole in parental species, F₁ hybrids and backcross. Left-hand graph: mean rate values. Right-hand graph: difference compared with controls on normal food. Vertical bars indicate the confidence intervals. sech, *D. sechellia*; BC sech, backcross with *D. sechellia*; F₁, first generation hybrids; BC sim, backcross with *D. simulans*; sim, *D. simulans*.

Table 3. Comparison of egg production traits in the two laboratory strains of *D. sechellia* collected in 1981 and 1985^a

Food	Strain	Ovariole number	Max. <i>F</i>	Rate	<i>n</i>
Normal food	81	17.1 ± 0.3	26.3 ± 1.0	1.5 ± 0.05	12
	85	17.3 ± 0.6	20.3 ± 0.5	1.2 ± 0.04	14
	Comparison (<i>t</i>)	0.2 ± 0.7 NS	6.0 ± 1.1***	0.3 ± 0.06***	
With morinda	81	18.2 ± 0.3	30.0 ± 0.6	1.66 ± 0.05	30
	85	18.3 ± 0.3	24.8 ± 0.6	1.37 ± 0.04	25
	Comparison (<i>t</i>)	0.1 ± 0.4 NS	5.2 ± 0.9***	0.29 ± 0.07***	
Difference morinda/normal food	81	—	3.7 ± 1.1**	0.16 ± 0.1 NS	
	85	—	4.5 ± 0.9***	0.17 ± 0.06**	

Max. *F*, maximum fecundity – the daily average over days 4–8; rate, maximum fecundity divided by the ovariole number; *n*, number of females.

Comparisons were by Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

^aThe experiment was done in 1989.

was also available. These two strains were compared in 1989, i.e. after 4 and 8 years of laboratory cultures (approximately 75 and 150 generations).

Comparisons were done both with normal food and with a small amount of morinda (Table 3). The ovariole numbers were similar in the two strains (17.89 and 17.94). On the other hand the maximum fecundity was significantly higher in the 1981 strain. Both strains reacted in a similar way to morinda. The difference arose mainly from a higher rate of oogenesis in the older strain, i.e. 1.5 vs 1.2 on normal food and 1.7 vs 1.4 on morinda. The exact cause of these differences is not known since no replicates were made. But we may suggest they arose as a consequence of laboratory culture. *D. sechellia* appears to be a very uniform and monomorphic species (Cariou *et al.*, 1990) and it is likely that the wild-living samples, collected in 1981 and 1985 in the same place, were genetically similar. Moreover, since the older strain exhibited a better reproductive potential, the difference is more likely to reflect some kind of adaptation to these new conditions than pure genetic drift.

4. Discussion and conclusions

The *D. melanogaster* species complex comprises four different species. Three of them are generalist (*D. melanogaster*, *D. simulans* and *D. mauritiana*) and use a diversity of resources. *D. sechellia* is strictly specialized on morinda, and exhibits several adaptive characteristics such as tolerance to fruit toxicity, adult attraction and female oviposition preference (R'Kha *et al.*, 1991).

D. sechellia is characterized by a very low reproductive potential of females, which explains why the species is difficult to keep in laboratory culture. In this particular case, it can be assumed that *D. sechellia* evolved in the Seychelles after a founder event, from a generalist ancestor. *D. sechellia* is distinguished from its generalist relatives (*D. simulans* and *D. mauritiana*)

by three characteristics: a dramatic reduction in ovariole number, a reduction (almost half) in the rate of oogenesis on normal food, and a stimulation of oogenesis when morinda is present, instead of an inhibition.

Egg production characteristics were investigated in F_1 and backcross progeny between *D. sechellia* and *D. simulans* (R'Kha *et al.*, 1991). Ovariole number varied in a purely additive manner, as also observed by Coyne *et al.* (1991). The high rate of egg production of *D. simulans* was, however, completely dominant. As might be expected, BC *simulans* females (i.e. from a backcross) were similar to pure *simulans*. Surprisingly, the BC *sechellia* were not intermediate between the F_1 and the parental species, but very close to *D. sechellia*.

From an evolutionary point of view, two kinds of related questions are raised: (1) How and why did these changes evolve? (2) Do they imply different genes or the same genes with pleiotropic effects? These two aspects will be discussed together.

The inhibition of oogenesis by morinda observed in *D. simulans* and *D. mauritiana* may be interpreted as a mere manifestation of morinda toxicity, which also kills the flies when the amount is increased. A genetic difference seems to exist, however, when adult survival and oogenesis rate are compared. For adult survival the sensitivity of *D. simulans* appeared as a recessive trait (R'Kha *et al.*, 1991), while for oogenesis the sensitivity of the same species is expressed in the F_1 as a dominant trait (see Fig. 4). The stimulation by morinda which is observed in *D. sechellia* may be considered as a specific trait, quite independent of its overall tolerance. The major argument is that stimulation was observed with a low quantity of morinda (70 mg) which was indifferent, i.e. non-toxic, to the sensitive species (see Fig. 3).

The likely adaptive scenario of *D. sechellia* on morinda is the following. Initially the sensitive generalist ancestor tried to breed on morinda but was

repelled or killed by the toxic products. On the other hand, fallen ripe fruits were progressively degraded by yeasts and bacteria so that, after a few days, the toxicity disappeared and sensitive larvae could develop. Indeed several sensitive species, including *D. mauritiana*, can be obtained from rotten morinda on Mauritius island (David *et al.*, 1989).

Since oogenesis took place on more or less rotten morinda, we may assume that the actual rate of oogenesis did not change very much over time. Initially the rate of the sensitive generalist was decreased from 2.0 to 1.5; then a progressive adaptation took place, fresher more toxic fruits could be used and the rate remained the same, and some physiological dependence (stimulation) developed in *D. sechellia*, in a parallel way to the overall tolerance. According to this scenario, the ovarian activity remained quite stable during the adaptive process without a significant decrease in fitness. It is likely that feeding on toxic morinda, even in the tolerant *D. sechellia*, exerts a permanent selective pressure for decreasing the rate of oogenesis. In the absence of morinda in the laboratory, this selection was released and resulted in a significant improvement in ovarian activity, presumably as an adaptation to the new, non-toxic environment.

The low ovariole number results in a major reduction in females fitness which is expressed under all circumstances. Ovariole number is an anatomical trait, determined during pupation, and for which a polygenic basis is known in various species of the *D. melanogaster* complex (Coyne *et al.*, 1991; Chakir *et al.*, 1995). In *D. melanogaster*, a comparison of various strains revealed a negative correlation between ovariole number and rate of oogenesis (David, 1970). The association in *D. sechellia* between a low rate and a small ovariole number is thus unexpected from a functional point of view. Moreover, there was apparently very little genetic variability for the latter trait, as shown by the lack of correlation between ovariole number and fecundity (Fig. 2) and the persistence of a low number in the laboratory-adapted strain. In that respect, *D. sechellia* appears to be very different from its relatives *D. melanogaster* and *D. simulans* (Capy *et al.*, 1994). Genetic analyses of various traits have demonstrated that *D. sechellia* harbours very little genetic variability, presumably related to a small population size (Cariou *et al.*, 1990). In this respect, some kind of inbreeding might explain the absence of variability. But the difficulty remains: Why did a smaller ovariole number progressively evolve during the speciation process? Two opposing hypotheses may be considered. The first is based on stochastic processes and repeated founder effects. *D. sechellia* exists on most islands in the Seychelles archipelago (unpublished observations), but presumably in small numbers. The population dynamics of its host plant is not well known but is presumably submitted to local extinctions and recolonizations, imposing the same metapopulation dynamics to *D.*

sechellia. During this process, extinctions and recolonizations might have fixed a small ovarian size. The second interpretation is an adaptive one: the low ovariole number, which is negatively related to fitness in laboratory conditions, might be positively correlated with fitness in nature if we take into account female survival. In *Drosophila*, ovarian development leads to a weight increase which certainly impairs flight capacities (David, 1979). Such excess weight might be counterselected by predators. It might also impair the dispersal capacities needed for finding breeding resources, which are often very dispersed. Finally, under the repeated foundations hypothesis, light females, with smaller ovaries, are likely to be more often involved in long-range dispersal than heavy ones.

In conclusion, the evolution towards a lesser female fitness which occurred in *D. sechellia* was apparently mediated by the need to use a toxic resource and to detoxify the food, by stochastic processes linked to a metapopulation dynamics, and possibly also by a strong selection on dispersal capacities. Also the toxic resource provided a protection against generalist competitors so that the selective pressure favouring a high reproductive potential was released. Field studies on this species should help to define these hypotheses better. From a physiological point of view, the various traits which distinguish *D. sechellia* from its generalist relatives apparently imply different functions, including anatomy, metabolic activity and hormonal secretions. Of course, these different functions might be determined by the pleiotropic effects of a few genes. It seems more likely, however, that various independent, sometimes polygenic genetic systems have been modified. Further investigations should help to solve this problem.

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