OVIPOSITION BEHAVIOUR IN FOUR SPECIES OF DROSOPHILA

CONDUCTA DE OVIPOSIÇãO EN CUATRO ESPECIES DE DROSOPHILA

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ABSTRACT

Oviposition site selection plays a central role in the evolutionary ecology of Drosophila, due to its high relation with the pre-adults fitness since the site selection determines the interactions between the species sharing common resources. Drosophila subobscura Collin which was detected in Chile for the first time in 1978, was found coexisting in the wild, as adults, over apple and raspberry, with D. hydei, D. immigrans and D. melanogaster. It was observed that raspberry has been used only by Drosophila subobscura as a breeding site; meanwhile sharing apple with the others. Our principal purpose was to shed light on the ecological significance of the new species oviposition behaviour; analysing also some of the preference-performance relationship. Two experiments of non-choice and choice, were carried out. The results showed that all species used both substrates as a breeding site. However, D. subobscura and D. melanogaster chose significantly raspberry, whereas D. immigrans and D. hydei preferred apple. In relation to preference-performance, only D. melanogaster expressed that positively in terms of short development time and high viability. The physical characters of the substrate could explain the oviposition preference. These results constitute the first empirical evidence that D. subobscura is constantly colonising new breeding sites, if it is known that raspberry has been cultivated for one decade.

KEYWORDS: Oviposition behaviour, D. subobscura, colonisation, preference - performance relationship, and substrate physical characters.

INTRODUCTION

The oviposition site selection in Drosophila, among many other ecological and behavioural factors, plays a central role in the community structure. Being immobile, pre-adults instars are directly influenced by the decision taken by their mothers where to lay their eggs because it...
determines their fitness especially when sharing resources with other species. The species of *Drosophila* are generally selective in their selection of breeding site. As a species in process of colonisation of a new habitat, *Drosophila subobscura* Collin (Diptera: Drosophilidae) is using new breeding sites in Chile to avoid competitive interactions in nature, a strategy proposed by Budnik and Brncic (1983) who found in experiments of competition performed in the laboratory, using artificial culture medium, that *D. subobscura* interferes with *D. simulans, D. immigrans, D. pavanii* as well as with *D. melanogaster*. *D. subobscura* is a typically Paleartic species distributed all over Europe, North Africa and Asia Minor, with the exception of the northern parts of Scandinavia and Finland (Lakovaara and Saura 1982). The species was detected in Chile for the first time in Puerto Montt (41°60'S) in 1978 (Brncic and Budnik 1980). In the next year the insect expanded its range from Puerto Montt to La Serena (29°55'S) where its frequency in relation to the total collected drosophilids was 49.09 and 0.45%, respectively (Budnik and Brncic 1982). The species was found far southern as Punta Arenas (54°40'S), with a frequency of 1.4 (Budnik and Brncic 1982). The insect has also crossed the Andes mountain range and was detected in the Pacific coast of North America, where it has now become established (Prevosti et al. 1982).

Samples of *Drosophila* collected in southern Chile (Temuco and Valdivia) by Videla et al. (1994) showed that adults of *D. hydei*, *D. immigrans, D. melanogaster* and *D. subobscura* coexist over decayed fruits of apple, peach, plum, and raspberry; however, in the laboratory, it was seen that from the first three fruits, emerged all the four species while from raspberry only *D. subobscura* did. So, considering these findings, the aim of this paper was to shed light on the ecological significance of oviposition site selection by *D. subobscura* as a new species colonising Chile, as well as *D. hydei, D. immigrans*; and *D. melanogaster* in two natural substrates of raspberry and apple.

Furthermore, the study aims to evaluate preference - performance relationship in terms of the time needed to reach the adult stage and the viability.

This work would try to find answer to the two specific questions; does raspberry offer a suitable breeding site for *D. subobscura*? Should other females lay their eggs on raspberry, would the egg-to-adult development time and viability be affected in comparison to other substrate where they been?

**MATERIALS AND METHODS**

Two oviposition experiments were conducted without and with substrate choice. The population of the four species used in the study were derived from flies captured in the university experim station located at the north of Valdivia (39° 73°14'W). The species were reared in 250-cm3 bottles using 50 cc of Burdik culture medium (Burdik 1954). The fruits offered were apple (*A. sylvestris, CV. Granny*), and raspberry (*R. idaeceus CV. Heri ge*), which were healthy untreated, collected one day before the beginning of the experiments.

**EXPERIMENT I. NON-CHOICE EXPERIMENT**

Transparent acrylic population cage 35x35x11cm, containing twenty-five 2x2 oviposition glass tubes arranged in five rows five columns, were used. Variable quantity of river sand was added to the oviposition tubes in a way that the substrates maintain the same level, then 4.5 ± 0.1 gr. of apple piece or 2.5 gr. of raspberry fruit was placed on the sterilised filter paper was added to the sand to that females lay eggs on the sand. Afterward fruit surface was sprayed with Nipagin 1% to prevent the bacterial and fungal contamination.

After leaving 25 females of maxi fecundity age of each species, introduced with anaesthesia, for 24 h in the cage, the tubes withdrawn and the number of eggs, if any examined. Those containing eggs were incubated to be checked daily for the insect presence until week after the emergence of the last adult experiment starts at 10:00 A.M., and the withdrawal process is done at 10:00 A.M. the following day.

Five replicates for each species and substrates were used.

**EXPERIMENT II: CHOICE EXPERIMENT**

The whole procedure used was similar to the previous experiment, with the except offering simultaneously 12-apple oviposition areas and 12-raspberry oviposition distributed randomly in the population cage.
Five replicates for each species were carried out.

The experiments were done in a controlled room of 22 ± 1°C, 60% R.H and 12L: 12D (08:00-20:00).

RESULTS

NON CHOICE EXPERIMENT

First of all, the most remarkable fact, as can be seen from Table 1, was that all species oviposited on both substrates; however, significant differences were found between species (ANOVA and a posterior "Hikey's test) within each substrate. In raspberry, for example, *D. subobscura* laid significantly more eggs than did the other three species (*F*<sub>3,16</sub> = 13.3; *P*< 0.001) and occupied more available oviposition areas (*F*<sub>3,8</sub> = 13.5; *P*< 0.001). Meanwhile, in apple, the situation is completely opposite in the sense that *D. subobscura* had the less number of eggs and occupied less tubes in comparison to *D. hydei*, *D. melanogaster* and *D. immigrans*. It seems there is an apparently proportional relationship, in the case of *D. subobscura* between the number of tubes occupied and the number of eggs laid, on the contrary of the other species. As for spatial egg distribution, it was clear that all species are gregarious, since the variance-mean ratio was more than one, irrespective of the substrate, egg number, and tubes amount.

| Species       | Substrate | Eggs number | Tubes number | Aggregation's rate |
|---------------|-----------|-------------|--------------|--------------------|
| *D. hydei*    | Raspberry | 84.4 ± 19.0 | 8.4 ± 0.9    | 7.1 ± 2.1          |
| *D. immigrans*| Raspberry | 171.2 ± 35.1| 12.2 ± 1.9   | 4.8 ± 0.7          |
| *D. melanogaster* | Raspberry | 173.8 ± 22.7| 14.0 ± 1.6   | 5.5 ± 1.2          |
| *D. subobscura* | Raspberry | 342.4 ± 37.5| 20.4 ± 0.8   | 7.1 ± 1.4          |
| *D. hydei*    | Apple     | 233.8 ± 43.7| 17.8 ± 1.6   | 9.7 ± 1.4          |
| *D. immigrans*| Apple     | 153.0 ± 29.5| 18.2 ± 3.9   | 4.6 ± 0.5          |
| *D. melanogaster* | Apple     | 191.4 ± 44.1| 18.8 ± 1.1   | 5.5 ± 1.8          |
| *D. subobscura* | Apple     | 81.0 ± 14.9 | 11.8 ± 0.9   | 2.8 ± 0.7          |

Values in each column with the same letter are not significantly different at the 5% level.

CHOICE EXPERIMENT

*D. subobscura* as well as *D. melanogaster* preferred ovipositing on raspberry (x²=47.0; x²=426.0; 1 df; *P*< 0.001, respectively), meanwhile *D. hydei* and *D. immigrans* laid their eggs on apple (x²=51.5; x²=118.8; 1 df; *P*< 0.001, respectively).

| Species       | Raspberry | Apple | x²   | d.f. = 1 |
|---------------|-----------|-------|------|---------|
| *D. hydei*    | 42.0 ± 15.9| 77.0 ± 22.6| 51.5 | ***     |
| *D. immigrans*| 40.0 ± 15.6| 177.0 ± 9.8| 118.8| ***     |
| *D. melanogaster* | 201.0 ± 16.9| 53.6 ± 8.9 | 426.0 | ***     |
| *D. subobscura* | 130.5 ± 24.6| 85.4 ± 27.2| 47.0  | ***     |

With regards to the two parameters used to measure, if any, the preference-performance relationship, i.e., development time of egg-to-adult and mortality; Table III indicates significant differences between species in both substrates and sexes as *D. melanogaster* being the species that reached the adult stage in short time (ANOVA and a posterior Tukey's test). In raspberry, for example,
D. melanogaster took less development time expressed in days (F \(_{3,118}\) = 136.3; P < 0.0000 and F \(_{3,92}\) = 55.7; P < 0.0000, for females and males, respectively). It is worthy to mention that only D. melanogaster (F = 3.64, with 4 df; P < 0.04 and F = 3.19, with 4 df; P < 0.03, for females and males, respectively) had the positive preference-performance relationship where raspberry was used as a breeding site (T III, T-test).

**Table III. Development time of females and males (in days) showing the relationship of preference - perform (Means ±SE).**

| Species       | Females development time | T-test | Males development time | T-test |
|---------------|--------------------------|--------|------------------------|--------|
|               | Raspberry | Apple |                      | Raspberry | Apple |        |
| D. hydei      | 20.8±1.5 \(b\)  | 19.7±0.3 \(*\) | n.s | 18.0±0.0 \(*\) | 19.5±0.4 \(*\) | n.s |
| D. immigrans  | 15.6±0.7 \(b\)  | 16.8±0.2 \(b\) | n.s | 15.3±0.7 \(b\) | 17.3±0.3 \(b\) | n.s |
| D. melanogaster | 11.9±0.2 \(c\) | 14.9±0.9 \(*\) | 3.64\(*\) | 12.5±0.8 \(c\) | 16.3±2.2 \(b\) | 3.19\(*\) |
| D. subobscura | 18.8±0.2 \(*\) | 18.7±0.3\(*\) | n.s | 9.1±0.3 \(*\) | 18.6±0.3 \(*\) | n.s |

Values in each column with the same letter are not significantly different at the 5% level.

It is interesting to observe from graphics 1 and 2 that D. melanogaster was the only species that had the high percent of emerging individuals (females and males) in both substrates, i.e., the preferred as well as the non-preferred. On the other hand, D. subob. and D. hydei showed their high percentage of individuals in the preferred substrate only; while D. immigrans did that in the non-preferred substrate.

![Graphic 1. Accumulative emergency in raspberry.](image-url)
With regards to mortality, both *D. melanogaster* in raspberry and *D. immigrans* in apple, had such relationship (Table IV, T-test), as the ANOVA and a posterior Tukey’s test indicate significant differences between species only in raspberry ($F_{3,16}$ = 5.1; $P<0.01$).

**Table IV.** Percentage of total mortality between species expressed by ANOVA and T-test (Means ±SE).

| Species      | Raspberry | Apple | T-test |
|--------------|-----------|-------|--------|
| *D. hydei*   | 65.5±16.5 | 53.9±5.3 | n.s    |
| *D. immigrans* | 77.3±5.8  | 50.1±5.6 | 2.97*  |
| *D. melanogaster* | 29.8±1.8  | 62.1±10.9 | 2.73*  |
| *D. subobscura* | 57.1±2.4  | 53.2±5.8 | n.s    |

Values in each column with the same letter are not significantly different at the 5% level.

**DISCUSSION**

The results of choice experiment will be discussed first because of its relevance to the ecology of the colonising species, i.e., *D. subobscura*. This helps to get some information on some of its behaviour patterns, i.e., oviposition preference, used by the insect in the new environment which somehow will provide the necessary steps needed to establish well. Then I proceed to deal with the first experiment. The substrate physical conditions such as shape, texture and colour could explain the results obtained. It seems that the convex shape of raspberry was used as a positive visual stimulus in comparison to the flat. Such finding was assured by Ruiz *et al.* 1994, that *D. melanogaster*, in front of concave and convex surfaces (culture medium), preferred the later. In another work about *Chaetorellia australis* Hering
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(Diptera: Tephritidae), it was observed that the insect frequently visited spherical and conic substrates, and also it was mentioned that Ceratitis capitata (Wied) (Tephritidae), preferred convex rather than plane or concave (Pittara 1992). Degen & Städler (1996) observed that Delia antiqua (Meigen) (Diptera: Anthomyiidae) did not lay significantly more eggs around the host leaf which was flat and too wide at the base rather than cylindrical and narrow like its main host plant, the onion (Allium cepa).

Referring to substrate texture, the structural arrangement of the fruit drupes as well as the spaces between them, had an attractive effect for D. subobscura to choose in the sense that the texture might provided the protection for the eggs; besides, it might help the females perceive and detect olfactory stimulus through antenna or rather by chemical ones located in the tarsus when the insect is going up and down the fruit. Once the insect landed on the fruit surface, it begins and exhibits a series of preovipositional activities that lead finally to accept the substrate as a site for the deposition of eggs (Harris & Miller 1984).

Results found by Prophetou-Athanasiadou et al. (1993) showed that D. subobscura used significantly the more rough surface, out of three offered surfaces with different roughness, laying there more eggs.

Many researchers (Atkinson & Shorrocks 1981) indicated the tendency of D. melanogaster to exhibit a significant preference to the rough surface as a site of oviposition and this observation or fact was used then by Atkinson 1983 to argue that texture irregularity is the factor that expresses the gregarious behaviour in this species.

Chess & Ringo (1985), demonstrated that D. melanogaster females with amputated tarsus and antenna, continued laying eggs on the rough substrate in a proportion reached to 90% than on smooth surface. In addition, it was observed that D. simulans, preferred to oviposit in the centre of the culture medium which has more roughness than the border (Grossfield 1983).

Roessingh & Städler (1990), mentioned that Delia radicum (L.) (Diptera: Anthomyiidae) in the natural as well as in the artificial substrates, always used the irregular surface.

It was observed in this study that both D. subobscura and D. melanogaster, frequently lay their eggs on the border and inside the fruit which probably provide a suitable site that is their pre-adult instars development. Sirvast Singh (1983) also noticed such behaviour in Indian species of Drosophila. This observation be attributed partially to the humidity content border and the inside are more wet and consequently more suitable than the outside for the growth; observation agrees with the work of Chess & Städler (1985) that D. melanogaster lay more eggs on than on dry part of the substrate.

Another factor that might be involved in site selection, is the colour, as there are evidences in the Diptera, including Droso indicate the preference for a certain colour. This evidence was presented by Carfagna & Le (1971), who declared that there is a cause-relationship between the extraretinal pigments frequent oviposition on determined colour melanogaster. Del Solar et al. (1974, 1976) in a selection experiment that D. melanogaster females preferred coloured areas than non-coloured areas and preferred the orange coloured substrates.

As for the preference exhibited by D. and D. immigrans to the flat and smooth sul that is similar to the culture medium where were cultivated; Del Solar & Ruiz (1979) not D. melanogaster females prefer to lay eggs in that area similar to that one where they had been in relation to colour and superficies. These s in the nature emerged frequently from apple (et al. 1994).

With relation to non-choice experiment all species laid eggs on raspberry as well as on the following explanation could account for results obtained. The insect will lay its egg on certain host when the stimulus received from potential host plant exceeds some threshold this threshold is variable and decreases as the time increases and for that reason the insect accept the less preferred host, although th
affect development of the insect (Jaenike 1990 and references therein).

In this regards, it is necessary to take into consideration, on analysing such complicated behaviour, the multiple stimulus and not to overestimate one factor per se (Deither 1982).

PREFERENCE - PERFORMANCE RELATIONSHIP.

Only D. melanogaster, both females and males, showed positive relationship in the parameters used, development time as it took short time to the adult stage as well as the high viability. This could be principally explained by the fact that in raspberry, the feeding rate due to the liquid content of the fruit is so high and the insect has a fast search capacity (Burnet et al. 1977) and then getting the food more easily without spending more effort. Besides, D. melanogaster as a species, is known by its evolutionary history to decrease its body size in order to lessen as possible the mortality even if it encounters intraspecific conditions (Miller 1964). Many researchers had indicated that development time in drosophilids, depends principally on food consumed as insects need to reach the minimum size required during the third instar, before completing growth to become pupa (Robertson 1963; Miller 1964; Budnik 1977; Barker 1992). On the other hand, in the case of D. immigrans, this relationship is partially expressed by the relatively low mortality in apple in comparison to raspberry as might be explained by the insect large size. The other species, D. hydei and D. subobscura, failed to display this tendency. It is worthy to mention that this relation is not a direct consequence of plant-insect relationship, for example, Kearney (1983), found that D. subobscura although preferred Rubus fructicosus L. (blackberry) but relationship was observed partially in terms of body size which the present study did not measure.

This relationship range from positive (Singer 1983; Singer et al. 1988; Criage et al. 1989; Dodge et al. 1990; Ryoo & Cho 1992; Waddell & Mousseau 1996) to negative (Courtney 1981; Karban & Courtney 1988; Auerbach & Simberloff 1989; Fox & Lalonde 1993; Ahman & Lovgren 1995), as for example Larson and Strong (1992) had revealed that Dasineura marginemtorquens (Diptera: Cecidomyiidae) preferred oviposit on Salix viminalis, in spite of being resistant to the attack to the extent that at the sixth day of development, the insect survival was in the terms of just 6%.

Finally, it would be necessary to explain some of the ecological significance of oviposition behaviour showed by D. subobscura in the sense of implications that could be drawn about the insect process of colonisation which implicitly include the probable interactions with other species in terms of utilising food resources (including breeding sites) and consequences that might result from such interactions in the long run. The results presented here agree well with those observed in the nature, which indicate that as a new species, the insect is always seeking new sites not used by other, specially if we know that raspberry has been a recently introduced and cultivated in southern Chile. As Budnik & Brncic (1983) postulated that D. subobscura, being inferior in its competitive ability, and to avoid or escape the negative effects it should use new habitats in its new environment.

For example it was found in the wild native trees like Berberis buxifolia, Maytenus magallanica, Embothrium coccineum and various species of Nothofagus. So, the results of this study represent as far as it is concerned to the ecological implication of the insect behaviour, the first empirical findings supporting and confirming the above-mentioned hypothesis, and also confirm its poor competitive ability, since the development time, body size and viability as fitness components, were negatively affected in competition experiment with the established species in Chile in the culture medium experiments (Budnik & Cifuentes 1989,1993) as well as in natural substrates ( Alamiri 2000).

As a successful coloniser, the insect must be less adapted to the particular local environments of the territories, i.e., be able to occupy a contrasting climates where there may be an association between the level of chromosome polymorphism and ecological phenotypes. (Parsons 1982 and references therein). This is clearly evident as the insect had extended its distribution on 3000 km north-south gradient ranging from semi-arid zones to wet and cold zones (Budnik & Brncic 1982), beside other characters such as the rapid differentiation of its populations here in Chile as compared to the European populations which was proved by analysis of chromosomal and enzymatic polymorphism (Brncic et al. 1981; Brncic & Budnik 1987; Prevosti et al. 1985, 1987) and quantitative characters relevant to the microdifferentiation process, such as sexual isolation i.e., interpopulation differences in mating (Brncic & Budnik 1984).