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PREADULT INTERACTIONS BETWEEN *DROSOPHILA SIMULANS* AND *D. WILLISTONI* (DIPTERA: DROSOPHILIDAE) EMERGED FROM THE SAME SUBSTRATA

INTERACCIONES PREADULTAS ENTRE *DROSOPHILA SIMULANS* Y *D. WILLISTONI* (DIPTERA: DROSOPHILIDAE) EMERGIDAS DEL MISMO SUBSTRATO

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ABSTRACT

In samples of *D. willistoni* and *D. simulans* emerging from individual fruits, the effect of larvae metabolic waste products on viability and egg to adult developmental time (in days) was studied. Different stocks were established with the flies emerged from individual fruits. The collections were performed in Southern Brazil. These parameters are significantly altered when larvae are bred in a culture medium where heterospecific preadults of different stocks of either, *D. willistoni* or *D. simulans*, have developed previously. The viability of both species was reduced and the development time of *D. simulans* was enlarged with the waste metabolic products of *D. willistoni*. The response varies between species and between stocks within species. We discuss this finding in terms of the relative importance of such kind of interactions in the coexistence or exclusion of species in nature.

KEY WORDS: *D. simulans, D. willistoni*, preadult interactions, viability and preadult development time, waste metabolic products.

INTRODUCTION

The Neotropical *Drosophila* (Diptera, Drosophilidae) communities are particularly rich both in species number and in interactions (Dobzhansky &Pavan, 1950). Among the most common species in such communities, the native *D. willistoni* and the cosmopolitan *D. simulans* are frequently associated in the exploitation of a wide range of trophic resources as feeding and/or breeding sites (Dobzhansky & Pavan, 1950; Pavan, 1952; Brncic & Valente, 1978; Araujo & Valente, 1981; Valente & Araujo, 1985, 1991).

In spite of being highly coincident in their substrate preferences, as detected by several authors in very different neotropical latitudes, *D. willistoni* and *D. simulans* apparently do not compete, showing even some evidence for a successful niche partitioning (Dobzhansky & Pavan, 1950; Valente & Araujo, 1985, 1991). Several explanations have been given, like their coexistence due to differential use of microorganisms growing in the same fruit substratum (Da Cunha, 1957). However, it is difficult to imagine that such a close neighborhood would not have deleterious effects on their life cycle and other parameters (Valente & Araujo, 1985, 1991).

In various species of *Drosophila*, development time as well as egg-to-adult viability are altered when preadults of a species develop in a medium which has been used previously by homospecific larvae, or by larvae of different species ("conditioning" of the medium) (Weisbrot, 1966; Dawood & Strickberger, 1969). A more important consideration is that these effects occur in the absence of resource limitation

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(Budnik & Brncic, 1976; Budnik, 1980; Budnik & Brncic, 1982; Barker, 1983; Budnik & Cifuentes, 1993). A possible explanation for this phenomenon has been the accumulation of larval metabolic waste products which might either interfere with, or promote the growth of yeast and other food resources of *Drosophila* larvae, thus affecting their survival and development (Weisbrot, 1966; Dawood & Strickberger, 1969).

The aim of the present study was to evaluate the effect of the accumulation of biotic residues of larvae in absence of resource shortage on the preadult survival and development time of samples of *D. willistoni* and *D. simulans* collected in South Brazil. The flies emerged of the same individual fruits. Such interspecific relationships could partially explain how certain species are able to live together in the same breeding sites, whereas others show clear tendencies to exclude each other. Thus, the null hypothesis predicts that viability and development time will be equal in the different samples under the effects of the waste metabolic products.

Material and Method

Rotten fruits of *Arecastrum romanzoffiana*, "coquinho", were collected according to a routine, which consisted in bringing to the laboratory this type of substrate supposed to contain eggs and larvae, and placing each fruit in vials with culture medium (Marques *et al.*, 1966), containing Baker's yeast diluted in water. After two weeks, the emerging adult flies were collected by aspiration, identified, sexed and counted. The total numbers of flies emerged were 387 *D. simulans* and 146 *D. willistoni*. All fruits collected had fallen on the ground around the same palm tree, in the Experimental Station of Guaiba, Eldorado county (30° 05' S; 51° 39' W), in the southernmost State of Brazil.

All *D. simulans* and *D. willistoni* emerging from each fruit originated the stocks of each species had been kept in the laboratory in mass cultures in bottles with culture media during a few months previous to their use in these experiments. Thus, 4 stocks of *D. willistoni* and 4 stocks of *D. simulans* were established.

The procedure used to investigate the effects of larval waste products on the viability and egg-to-adult development time (in days) was similar to that used by Weisbrot (1966), Budnik & Brncic (1983) and Budnik & Cifuentes (1993, 1995). Fifty fertilized eggs of the same age belonging to each stock of *D. willistoni* and *D. simulans* were transferred to 7 x 3 cm tubes containing 10 cc of basic culture medium for *Drosophila*. These were stored in a constant temperature chamber at 22° C and larvae were allowed to develop for three days and then killed by a temperature shock of -30° C lasting 6 hours. Then, the tubes were left in a 22° C chamber for 24 hours in order to defrost. The culture media thus treated became "conditioned". The vials conditioned by the 4 stocks of *D. willistoni* received 20 eggs of *D. simulans* each, and those "conditioned" by the 4 stocks of *D. simulans* received 20 eggs of *D. willistoni* each. According to Lewontin (1955), Mather (1961), and others (Barker rev. 1983) this egg density corresponds to the optimal density which allows larval facilitation. The 50 larvae that conditioned the food medium came from the same coquinho as the 20 eggs of the other species. The total number of eggs sown in each experimental group was 1000 (50 tubes). The control groups consisted of series of 50 vials with fresh "non conditioned" culture media into which 20 fertilized eggs of each stock of *D. willistoni* or *D. simulans* were sown. These vials were frozen and defrosted just as those of the experimental groups.

All vials were kept in the constant temperature chamber at 22° C. The emergence of adults was counted daily. Experiments in homospecific conditions in *D. willistoni* and *D. simulans* have been done previously and they showed no interference on viability (Budnik & Brncic, 1976; Budnik, 1980).

The mean differences of viability and pre-adult development time between "conditioned" and "non-conditioned" groups were estimated. The effects of conditioning on viability and development time in relation to species and stock within a given species were evaluated by means of a nested analysis of variance. The various a posteriori comparisons were made using the Tukey-T-Test. Statistic analyses were performed by means of the SAS sub-routines (Allen, 1999).

Results

Table 1 shows preadult viability expressed as the mean emergence per vial together with the corresponding standard errors. It is interesting to point out that viability decreases in all conditioned groups, and that when compared to the corresponding control groups, differences between means are significant, with the

TABLE I

MEAN ($\bar{X}$) AND STANDARD ERRORS (S.E.) OF VIABILITY AND EGG-TO-ADULT DEVELOPMENT TIME (IN DAYS) OF 4 STOCKS OF *D. SIMULANS* AND *D. WILLISTONI* PROCEEDING FROM 4 FRUITS OF *ARECASTRUN ROMANZOFFIANUM* "COQUINHO" BRED AT 22° C IN "NON CONDITIONED" AND "CONDITIONED" CULTURE MEDIA WITH 30 *D. WILLISTONI* OR *D. SIMULANS*. THE VIALS CONDITIONED BY THE 4 STOCKS OF *D. WILLISTONI* RECEIVED 30 EGGS OF *D. SIMULANS* EACH, AND THOSE CONDITIONED BY THE 4 STOCKS OF *D. SIMULANS* RECEIVED 30 EGGS OF *D. WILLISTONI* EACH. EACH GROUP CONSISTED OF 30 VIALS. THE NON CONDITIONING GROUP CONSISTED OF SERIES 30 VIALS INTO WHICH 30 FERTILIZED EGGS OF EACH STOCK OF *D. WILLISTONI* OR *D. SIMULANS* WERE SOWN.

STOCKS	CULTURE MEDIA	VIABILITY PER VIAL ($\bar{X}$ ± S.E.)	DEVELOPMENT TIME (IN DAYS) ($\bar{X}$ ± S.E.)
D. willistoni			
coquinho 1	Non conditioned	13.30 ± 0.34	12.68 ± 0.02
	conditioned (with *D. simulans*)	12.34 ± 0.31	12.12 ± 0.03**
coquinho 2	Non conditioned	11.70 ± 0.39	13.07 ± 0.02
	conditioned (with *D. simulans*)	10.78 ± 0.42	12.98 ± 0.05
coquinho 3	Non conditioned	14.60 ± 0.27	12.39 ± 0.02
	conditioned (with *D. simulans*)	11.76 ± 0.41*	12.84 ± 0.04**
coquinho 4	Non conditioned	8.92 ± 0.40	12.27 ± 0.03
	conditioned (with *D. simulans*)	2.98 ± 0.63*	12.80 ± 0.09**
TOTAL	Non conditioned	12.13 ± 0.24	12.61 ± 0.01
	conditioned (with *D. simulans*)	9.32 ± 0.35*	12.64 ± 0.02
D. simulans			
coquinho 1	Non conditioned	16.62 ± 0.27	12.12 ± 0.01
	conditioned (with *D. willistoni*)	14.84 ± 0.43*	10.38 ± 0.03**
coquinho 2	Non conditioned	11.28 ± 0.30	12.17 ± 0.02
	conditioned (with *D. willistoni*)	9.76 ± 0.43*	10.44 ± 0.04*
coquinho 3	Non conditioned	16.46 ± 0.23	12.10 ± 0.01
	conditioned (with *D. willistoni*)	15.46 ± 0.35*	11.16 ± 0.02**
coquinho 4	Non conditioned	11.06 ± 0.33	10.50 ± 0.03
	conditioned (with *D. willistoni*)	4.88 ± 0.73*	11.19 ± 0.06**
TOTAL	Non conditioned	13.86 ± 0.23	11.80 ± 0.01
	conditioned (with *D. willistoni*)	11.24 ± 0.39*	10.75 ± 0.02**

* $P \leq 0.05$ (∞ d.f.) ** $P \leq 0.01$ (∞ d.f.) test-t

exception of the *D. willistoni* stocks derived from coquinho 1 and 2. However, the magnitude of these differences, as well as viability, differed according to the species or stocks. The nested analysis of variance showed that the effect of larval detritus on viability of species, stocks within a given species, and of conditioning, were statistically significant (Table 2). Multiple comparisons between the different stocks within both species by means of the Tukey Test, preadult viability was found to be different in all stocks with the exception of the stocks from coquinho 1 and 3. Further, non-parametric analyses of variance were performed (Kruskall-Wallis Test) in order to assess the independent effect of the different factors on preadult viability. The totality results agrees with those obtained in the parametric analysis (Table 2).

Table 1 also shows that the modifications of egg-to-adult development time in the "conditioned" groups and the differences with the corresponding "non-conditioned" ones, were statistically significant, excepting the stock of *D. willistoni* from coquinho 2. The nested analysis of variance also showed that the factors species stock within a given species, and conditioning, significantly influenced development time (Table 3). When performing multiple comparisons between the different stocks within both species by means of the Tukey Test, development times differed in all stocks with the exception of the stocks of *D. willistoni* from fruits 1 and 4 and the stocks of fruits 1 and 2 of *D. simulans* which did not differ. An additional non parametric analysis of variance (Kruskall-Wallis) was also performed in order to evaluate

TABLE II

ESTIMATION OF THE EFFECT OF SPECIES, STOCKS AND CONDITIONING FACTOR ON PREADULT VIABILITY PER VIAL IN 4 STOCKS OF *D. SIMULANS* AND *D. WILLISTONI* BY MEANS OF A NESTED ANALYSIS OF VARIANCE.

Source of variation	f.d	SS	SSM	F
Species	1	593.40	593.40	67.60*
Stocks within species	6	8071.06	1345.18	153.25*
Conditioning within stocks	4	2175.57	543.89	61.96*
Error	788	6916.97	8.78	
Total	799	17756.99		

* P < 0.0001

TABLE III

ESTIMATION OF THE EFFECT OF SPECIES, STOCKS AND CONDITIONING FACTORS ON DEVELOPMENT TIME (IN DAYS) IN 4 STOCKS OF *D. SIMULANS* AND *D. WILLISTONI* BY MEANS OF A NESTED ANALYSIS OF VARIANCE.

Source of variation	f.d	SS	SSM	F
Species	1	3892.44	3892.44	5296.08*
Stocks within species	6	731.97	121.99	165.99*
Conditioning within stocks	4	1651.10	412.77	561.62*
Error	9336	6861.64	0.74	
Total	9347	13137.12		

* P < 0.0001

separately the effect on this parameter of the different factors studied. The results obtained matched those obtained with the parametric tests previously described.

Discussion

Although apparently there exists a great variety of available resources for polyphagous species in nature, it should be considered that Drosophilidae present a strong gregarious tendency for egg laying (Barker, 1983; Del Solar *et al.* 1985; Brncic, 1987). As a consequence, preadults would be submitted to competitive interactions and also to the action of larval catabolites during development.

These experiments showed that viability and egg-to-adult development time (in days) of 4 stocks of *D. simulans* are modified when their preadults are bred in media conditioned by the other species; and the response magnitude in each stock within each species is different.

D. willistoni and *D. simulans* frequently emerge together from the same fruits coming from very different plants and places. In both species, specially in the various stocks of *D. willistoni*, the detritus of the other species decreases preadult viability. This decrease of viability has also been observed in flies emerging from fruits collected in their natural habitats (Araujo & Valente, 1981; Valente & Araujo, 1985). The effect on development time is often significant but varies in direction. Sang (1949) showed that "larvae metabolic products do not impede but may even encourage larvae development". In some groups, development time is shortened. This fact may indicate an adaptive advantage in the coexistence of both species, because larvae are more likely to complete development before their resources patch is exhausted (Nunney, 1990; Sevenster & Van Alphen, 1993).

This finding would partially explain that *D. willistoni* and *D. simulans* might obtain some advantages in their coexistence that could compensate for the stock's decreased viability when larvae of the other species are present in the breeding sites. However we must be cautious in the interpretation of theses results under laboratory experimentation, because they did not reflect entirely the conditions existing in the natural substrates utilized by the species.

The mode of action of detritus as well as the nature of the chemical substances they contain is still unknown. In recent years we have collected some data jointing to biotic residues depends on the species or genotypes involved, and on the stage of development at which conditioning acts (Budnik & Brncic, 1976, 1983; Budnik, 1980; Budnik & Cifuentes, 1993). The preadult viability of some chilean endemic species, such as *D. pavani*, is depressed by their own detritus, in contrast to *D. willistoni* and *D. simulans*, preadult viability is increased (Budnik & Brncic, 1976; Budnik, 1980). In *D. pavani*, the autodepression depends directly on detritus concentration (Budnik, 1977), a finding in strains of *D. melanogaster* (Hemmat & Eggleston, 1988). The toxic effect might be counterbalanced by the tunnels produced by larvae when conditioning the medium. A dilution of the toxic products on the surface of the substrate might occur, but an effect of facilitational intake from developing larvae may also be present (Budnik & Gajardo, 1981). The chemical nature of these substances is also unknown, but Botella *et al.*, (1985) demonstrated that urea and uric acid lengthen development time, reduce viability and that high doses arrest larval development of *D. melanogaster*. In unpublished studies Budnik and Morello have also found interference by urea in the preadult of *D. subobscura* and *D. pavani*. The reaction is species-specific and concentration-dependent. Budnik and Cifuentes (1995) have shown in the paleartic species *D. subobscura*, that colonized in Chile (Budnik & Brncic, 1982; Budnik *et al.*, 1997), modified their viability and preadult development of 8 stocks from different geographic zones when preadults were bred in homotipically conditioned environments without resource shortage. Further, the magnitude of the response varied according to the geographic origin of each stock. Similar result of interspecific competition was observed in these 8 stocks (Budnik & Cifuentes, 1995). Since Ricklefs (1989) has stated that each geographic population has its own genetic reservoir as a consequence of the specific history of local interactions, as well as of the local conditions of the environment. Barker (1983), Budnik *et al.* (1993, 1995) have proposed the need to consider interpopulational variations within a given species.

The present results could indicate that, in response to conditioning by larval detritus, not only the influence of the genotype of the species as a whole, or the genotype of the local population, would be determinant in larval viability and time of development, but also the degree of decomposition of the fruit in which the fly has developed. For further studies, it would be interesting to consider the degree of decomposition of the fruit at the time of establishment of the stocks in study, as well as the genetic structure of the individuals emerging in the media conditioned by these stocks.

It is not easy to find evidences for this kinds of competitive preadults interactions between species in nature. However, it is interesting to note that Brncic (1987) reported up to 4 *Drosophila* species emerging from a single natural fermenting fruit in an orchard in the out skirts of Santiago in Central Chile, and reported that *D. subobscura* was at a competitive disavantage in these natural fermenting fruits. Atkinson & Shorrocks (1977), Atkinson (1979), Nunney (1990), reported competitive interactions between species in nature.

The type of interaction described in the present study has also been observed in other insect species (Sokal & Sullivan, 1963; Karten, 1965). This is a complex phenomenon similar to the inhibitory effects of chemicals substances produced by plants which alter the development of other animal and plants (Allelopathy) (Price, 1975), but the relative importance of such kind of species interaction in nature must wait for further research.

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