

The effect of relative humidity and genetic strain on the interaction between *Drosophila hydei* and *D. melanogaster* in continuous culture.

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Introduction

Hodge and Mitchell (1998) demonstrated that atmospheric humidity and the water content of the resource could affect the interaction between *D. melanogaster* and *D. hydei* in single generation laboratory experiments. Previously, Arthur (1986) had shown that the interaction between these two species in continuous culture could be affected by the amount of resource, the mechanism of this effect being linked to smaller quantities of resource having relatively greater surface area to volume ratios and therefore being more prone to desiccation. Thus, the hydrological aspects of the environment appear to have an important role in determining how these flies interact.

The aim of this series of experiments was to further examine the effect of relative humidity on the interaction between *D. hydei* and *D. melanogaster* under conditions of continuous culture. In order to examine how general the effect of humidity is in affecting population performance and interactive outcomes, two different strains of *D. melanogaster* have been used.

Methods

Population cages consisted of clear plastic rectangular boxes (17 × 11 × 6cm) with six glass bottles (30ml) screwed into the underside. The two oldest bottles of medium were replaced each week, so each bottle of medium remained available to the flies for three weeks. Each glass bottle initially contained 1.5g of Instant *Drosophila* Medium (IDM; Blades Biological, Edenbridge, Kent) hydrated with 6ml of distilled water.

All experiments were carried out at 25±1°C and a light regime of 16:8 hours light:dark. Each experiment consisted of monoculture cages for each species and a mixed culture treatment. Populations were initiated using 50 males and 50 females. Cages at high humidity (45-50%) were maintained in an insect room. Cages designated to a low humidity (25-35%) were kept in an incubator, using trays of silica gel to maintain the low humidity. There were at least three replicates of each treatment.

At either 1-week or 2-week intervals, the whole adult population was anaesthetized using carbon dioxide and counted by hand. Every 8 weeks, the bottles of resource and adult flies were transferred to a clean cage. Populations were counted until at least week 12 or until two successive counts contained no adults.

Two different strains of *D. melanogaster* were used: one with a white eye mutation (*w*) and one carrying an ebony body (*e*) genetic marker. Both stocks were obtained from Phillip Harris Educational, Staffordshire, England, in 1993. The *D. hydei* stock was a wild-type strain derived from flies captured in Britain.

If a population became extinct, the equilibrium population size was designated as being zero. To estimate equilibrium values of extant populations, use was made of the negative relationship between growth rate and population size, characteristic of an S-shaped, logistic, growth pattern (Varley *et al.*, 1973). The *per capita* growth (PCG = $(N_{t+1} - N_t) / N_t$) was calculated for every two-week period, corresponding approximately to the generation time of the flies, and plotted against population size (N_t) (or the logarithm of population size, as the relationship was often curvi-linear) (Gilpin and Justice, 1972; Turchin, 1991). The regression equation produced was used to determine the population size at which *per capita* growth was equal to zero, *i.e.*, when the population was at a theoretical equilibrium. In most cases this method worked well, providing sensible values when comparing them to graphical representations of population time series. Data were analyzed using GLM, with relative humidity and presence/absence of the second species as factors.

Results

The average equilibrium population size for *D. hydei* was significantly greater at the higher humidity than at the lower ($P < 0.001$; Table 1), with populations becoming extinct in half of the cages maintained under low humidity conditions. The performance of *D. hydei* was not affected by either strain of *D. melanogaster* at either of the humidities used.

Table 1. Equilibrium population levels for *D. hydei*, *D. melanogaster w* and *D. melanogaster e*. (mean \pm SE). (*- indicates statistically significant difference from appropriate monoculture.)

Humidity	<i>D. hydei</i>			<i>D. mel w</i>		<i>D. mel e</i>	
	mono	mix (w)	mix (e)	mono	mix	mono	mix
Low	90 \pm 54	0 \pm 0	99 \pm 96	230 \pm 42	0 \pm 0*	317 \pm 161	473 \pm 84
High	313 \pm 41	263 \pm 40	274 \pm 47	164 \pm 14	1 \pm 1*	326 \pm 7	0 \pm 0*

Table 2. Summary of the interactions between different strains of *D. melanogaster* and *D. hydei* at different atmospheric humidities. (The symbol on the right represents the effect of *D. hydei* on *D. melanogaster*.)

Humidity		Strain of <i>D. melanogaster</i>	
		white eye	ebony body
Low (25-30%)	Low (25-30%)	(0, -)	(0, 0)
	High (45 - 50%)	(0, -)	(0, -)

D. melanogaster w performed similarly at each humidity (Table 1). *D. hydei* caused a significant reduction in *D. melanogaster w* population size at both humidities ($P < 0.001$), with *D. melanogaster w* being excluded in all but one mixed culture cage.

The equilibrium population size of *D. melanogaster e* responded significantly to

the interaction between relative humidity and the presence of *D. hydei* ($P < 0.02$; Table 1). At low humidity, *D. melanogaster e* was able to tolerate the presence of *D. hydei* and showed no difference in equilibrium population size. However, at high humidity the population size of *D. melanogaster e* was significantly reduced when *D. hydei* was present.

The pairwise interactions between *D. hydei* and *D. melanogaster* are summarized in Table 2. The interactions were predominantly amensal, with *D. hydei* being the dominant species. However, the interaction between *D. hydei* and *D. melanogaster e* at low humidity was neutral, the *D. melanogaster e* being able to coexist with *D. hydei* under these conditions. This implies that the interaction may or may not be perceived to change under different atmospheric conditions, depending on which strain of *D. melanogaster* is considered. Similarly, genetic strain may or may not influence the interactive outcome, dependent upon the humidity at which the experiment is performed.

Discussion

Relative humidity affects the interaction between *D. hydei* and *D. melanogaster* primarily through alterations in the performance of *D. hydei* (see Hodge, 1995; Hodge and Mitchell, 1998). The extinction of *D. hydei* in some low humidity cages was due to failure of the larvae rather than desiccation stress in the adult flies. Larval survival in *D. hydei* drops off rapidly if the resource is too dry (Hodge and Wilson, 1997) and resources which tend to dehydrate quickly can do so before the slow-developing *D. hydei* larvae complete their development (Arthur, 1986). At high humidity the resource remains fluid long enough to enable *D. hydei* larvae to grow and pupate. The rate of desiccation of the medium is an important factor in the success of *D. hydei* populations, both in monoculture and by affecting its competitive prowess in mixed cultures.

Differences found in the results of different experimenters working with *Drosophila* have sometimes been attributed to differences in the strain of flies used (e.g., Robertson and Sang, 1944) and *D. melanogaster* is known to possess different intra- and inter-strain competitive abilities (Lewontin, 1955; Bakker, 1961). Strains where adult flies have more pigmentation have been suggested as being more tolerant of dry atmospheres than paler flies (Kalmus, 1941) and this may explain why the white eye strain of *D. melanogaster* was unable to coexist with *D. hydei* in mixed cages at low humidity whereas the ebony body strain of *D. melanogaster* was able to persist.

The results of this experiment highlight once more that the environmental conditions under which populations are maintained in the laboratory can influence how they will interact in mixed cultures. The results also reinforce the need to take into account the genetic makeup of the populations used when considering the general applicability of results to other laboratory investigation and, ultimately, to a field situation.

References: Arthur, W., 1986, Philosophical Transactions of the Royal Society, Series B. 313: 471-508; Bakker, K., 1961, Archives Neerlandaises Zoology 14: 200-281; Gilpin, M.E., and K.E. Justice 1972, Nature 236: 273-274 and 299-301; Hodge, S., 1995, Interspecific facilitation in *Drosophila* systems. Unpublished PhD thesis, University of Sunderland, UK.; Hodge, S., and P. Mitchell 1998, Dros. Inf. Serv. 81: 131-133; Hodge, S., and N. Wilson 1997, The Entomologist 116: 93-103; Kalmus, H., 1941, Proceedings of the Royal Society of London, Series B 130: 185-201; Lewontin, R.C., 1955, Evolution 9: 27-41; Robertson, F.W., and J.H. Sang 1944, Proceedings of the Royal Society of London, Series B 132: 258-277; Turchin, P., 1991, Journal of Animal Ecology 60: 1091-1098; Varley, G.C., G.R. Gradwell, and M.P. Hassell 1973, *Insect Population Ecology, an Analytical Approach*. Oxford, Blackwell Scientific Publications.

The number and distribution of eggs laid by *D. melanogaster* and *D. hydei* is not influenced by the presence of the other species.

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Introduction

In nature, the interspecific interactions which take place within guilds of drosophilids are thought to be moderated by chance avoidance of species due to the independent aggregation of eggs between patches of resource (e.g., Atkinson and Shorrocks, 1984). *D. melanogaster* and *D. hydei* have been found to interact in various ways when maintained in laboratory systems (e.g., Arthur, 1986; Hodge and Mitchell, 1998; Hodge, 1999) but these previous experiments have generally been in the form of single generation experiments in glass tubes or have examined populations maintained in restricted culture cages. In the field, interactions between larvae may be abated or accentuated if females of one species tend to avoid or select sites occupied by the eggs of another. However, it is believed that the aggregated distribution of eggs produced by drosophilid females is produced independently of other species present in the guild (Rosewell *et al.*, 1990; Shorrocks *et al.*, 1990). This experiment examined how *D. melanogaster* and *D. hydei* interact, with respect to the number, distribution and success of eggs laid over an array of resource patches.

Methods

Experiments were carried out in 1.0m × 0.5m × 0.5m nylon mesh cages, maintained in an insect room at a temperature of 25°C, a light:dark cycle of 16:8 hours and a relative humidity of 35-45%. A 6 × 8 grid of small glass pots (30mm in diameter) containing food medium was placed in the centre of the cages on a plastic tray 35cm × 45cm. This gave a resource density of approximately 305 patches per m². Each pot contained 1.5g of Instant *Drosophila* Medium (IDM; Blades Biological, Edenbridge, Kent, UK) hydrated with 6.0ml of distilled water.

The populations used in the experiment were a white eye mutation of *D. melanogaster* Meigen and a wild-type strain of *D. hydei* Sturtevant. Single species cages were set up using 250 male and 250 female flies. Mixed culture cages used 250 male and 250 female flies of each species. Four replicates of each species and of the mixed culture treatment were set up.

The glass pots were removed from the cages after 18 hours, so the eggs could be counted before those of *D. melanogaster* started to hatch (the eggs of *D. melanogaster* and *D. hydei* are easily distinguished by the number of filaments). The degree of aggregation of eggs was estimated by calculating the variance / mean ratio and a value of *k* for a negative binomial distribution. The goodness-of-fit of the data to a negative binomial distribution was confirmed using a G-test.

The pots were then placed into individual plastic cups (9cm tall; 6cm diameter) with plastic screw lids. For ventilation, nine holes (4mm diameter) had been placed into the lids and then covered with nylon mesh. These cups were placed in an incubator set at 25°C, 16:8 hour light:dark cycle and a relative humidity of 35-45%. The cups were checked daily and any emerged adult flies removed.