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The development of photoresponse in *Drosophila melanogaster* larvae*

El desarrollo de la respuesta a la luz en las larvas de la *Drosophila melanogaster*

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ABSTRACT

Drosophila melanogaster larvae of 24 and 48 h developmental age show a preference for feeding in dark environments compared to those of 72 and 96 h post-hatching which remained in more illuminated zones. *D. melanogaster* larvae of the Oregon R-C, *vestigial* and *yellow* strains exhibit important differences in photobehavior at 24 and 96 h of age; these inter-strain differences tend to disappear in larvae of 48 and 72 h. Directional selection to increase and decrease the proportion of larvae remain in dark was applied at 24 and 72 h of larval of development. It was not possible to detect a response to selection for this character. Larval/adult mortality in the lines selected at 72 h was greater than in those selected at 24 h. The concepts of heritability and genetic homeostasis are discussed in relation to the responses to selection. Compared with those of the unselected population, larvae of lines selected at 24 h showed a different photobehavior at 24 and 48 h. At 72 and 96 h these larvae showed a similar photoresponse to those of the base population.

Key words: Selection, behavior, heritability, genetic homeostasis.

RESUMEN

Las larvas de *D. melanogaster* de 24 y 48 h de edad, prefieren alimentarse en ambientes oscuros comparadas con las de 72 y 96 h, las que permanecen en zonas más iluminadas. Las larvas de *D. melanogaster* de las cepas Oregon R-C, *vestigial* y *yellow*, exhiben diferencias importantes en fotocomportamiento a las 24 y 96 h de edad; estas diferencias entre cepas tienden a desaparecer en larvas de 48 y 72 h. Se aplicó selección direccional para aumentar y disminuir la proporción de larvas que permanecen en la oscuridad, a las 24 y a las 72 h de edad. No se obtuvo respuesta a la selección para esta conducta. La mortalidad larva/adulto en las líneas seleccionadas a las 72 h fue mayor que en aquellas seleccionadas a las 24 h. Se discuten los conceptos de heredabilidad y de homeostasis genética en relación con la respuesta a la selección. Comparadas con aquellas de la población no seleccionada, las larvas de la línea fotopositiva seleccionada a las 24 h de edad mostraron una fotoconducta diferente a las 24 y a las 48 h. A las 72 y a las 96 h estas mismas larvas mostraron una fotoconducta similar a la de la población base.

Palabras claves: Selección, comportamiento, heredabilidad, homeostasis genética.

INTRODUCTION

Epigenetic is a central concept in biology. New knowledge on the epigenetic of individual organisms may help to lay the foundations of a theoretical biology (Wilson 1981). Epigenetic changes have consequences for the development of behavioral patterns (Godoy-Herrera *et al.* 1984). Genetic studies on the development of behavior should help us to understand how heredity guides the maturation of a behavioral pattern (Connolly & Prechtl 1981, Caro & Bateson 1986). Individual differences in the development of a behavior may provide alternative solutions to the problem of adaptation to particular ecological conditions (Troncoso *et al.* 1987).

Movement patterns of insects are involved in their distribution in the wild (Andrewartha & Birch 1954, Brown 1975).

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* This paper is dedicated to Rosita Herrera-Sepúlveda.

The way in which an animal moves in its environment should contribute to maximize its fitness as a function, for example, of its feeding strategy, the search for conspecifics and avoidance of predators and/or parasites (Bond 1980). In particular, this kind of research could further our understanding of how *Drosophila* larvae perceive and react to changes in the temporal and spatial distribution of resources such as food and space (Parsons 1983). Additionally, this emphasis on how *Drosophila* larvae are adjusted to their natural breeding sites could also improve our understanding of the problems of behavioral adaptation to local environmental heterogeneity.

Few reports have been published on the development of orientation behavior in larvae of *Drosophila,* and information about the genetic contribution to such behavior is limited. Pruzan & Bush (1977) found that *Drosophila melanogaster* larvae may respond to residues of other conspecific larvae. Schnebel and Grossfield (1986) reported that, in the light, *Drosophila montana* larvae pupate near the surface of the food, whereas *D. melanogaster* pupates higher in light than in dark. Hutter (1986) observed that larvae of *D. melanogaster* and *Drosophila simulans* show preferences for pupation sites in a light gradient and that these preferences seem to be fitness-related. Mora & Godoy-Herrera (1986)[1] showed that larvae of *D. melanogaster* and *D. simulans* may orient themselves with respect to gravity depending on their age and genetic background.

The experiments reported in this paper were undertaken to explore the development of larval photoresponse of *D. melanogaster.* We examine this behavior at different stages of the life history of the larva and relate changes in larval photoresponse with epigenetic changes. We have chosen this larval behavior because it could have consequences for the distribution of groups of larvae with regard to feeding and pupation strategies and space utilization.

[1] MORA W & GODOY-HERRERA (1986). XIX Ann. Meeting of The Genetics Society of Chile.

MATERIALS AND METHODS

Stocks

The strains employed in these experiments were: Oregon R-C (wild type), *yellow (y)* and *vestigial (vg).* All stocks used were kept in half-pint bottles filled with about 50 cm of Burdick's medium (1954) at 24°C with a regime of 12 h of light and 12 h of dark.

The Oregon R-C strain also was used in the selection for positive and negative larval photoresponse. This strain is genetically heterogeneous for such larval behavior as burrowing into the culture medium (Godoy-Herrera, 1978).

Eggs and larvae collections

Groups of 60-80 inseminated females of the Oregon R-C, *yellow* and *vestigial* strains were left without food for a 3 h period in half-pint bottles. After this period we introduced into each one of the bottles a plastic spoon filled with medium spread with fresh yeast and acetic acid to stimulate oviposition. Eggs laid by the females were collected after 3-4 with the aid of a dissecting needle. About 50 eggs were incubated at 24°C and successive samples of larvae between 24-28, 48-52, 72-76 and 96-100 h of age were collected from the spoons. Each group of larvae was placed in a petri dish filled with 3% agar. After 5 min groups of 20 larvae were again captured and their photoresponse measured as described below.

Larval dispersal and photoresponse

Three 15 x 15 x 2 cm (width x length x height) perspex boxes were filled with 3% agar. After cooling, the agar was overlayed with a film of 2% yeast suspension in plain water (see Sewell *et al.* 1975). The yeast suspension inhibits the congregation of larvae at the edges or corners of the boxes, which occurs when *D. melanogaster* larvae disperse on an agar surface without food. The yeast suspension also inhibits the digging of larvae into agar or searching for scars or holes

in the substrate. These larval behaviors could confound our results. Each box was covered with a perspex lid. On the center of each lid, a thick, black paper disk of 8.5 cm in diameter was attached. The lid contributes to form a microenvironment in the boxes and prevents the light area of the boxes from being warmer than the dark area. This was important so that a temperature gradient did not exist at the dividing line. The boxes were illuminated by a white bulb of 60 watts placed 25 cm above them. In this way, each box had two different visual environments: a) a dark area produced by the shadow of the paper disk cast on the agar at the center of each box, and b) a more highly illuminated annulus surrounding the center disk. Larvae found in the dark zone presumably prefer a less illuminated environment for feeding (photonegative behavior); those found eating on the more illuminated area were classified as larvae showing a photopositive behavior.

Groups of 20 larvae between 24-28, 48-52, 72-76 and 96-100 h of age of the Oregon R-C, *yellow* and *vestigial* strains were deposited onto the agar of the boxes between the dark area and the illuminated area. The number of larvae in each visual environment was recorded in every 10 min up to 1 h. In other experimens, groups of 20 larvae of the same ages (24-28, 48-52, 72-76 and 96-100 h) for each one of the strains used were deposited either on the agar-yeast at the center of the dark area or in the more illuminated area 7.5 cm away from the center of the dark area. Again, the number of larvae in each visual environment was recorded every 10 min up to 1 hour. These experiments were controls for the first one in order to be sure we were measuring larval photoresponse. Each experiment was replicated 4 times.

Genetic selection for positive and negative larval photoresponse

Groups of 20 larvae at 24 and 72 h of the Oregon R-C strain were each deposited on agar of 3 boxes between the dark area and the more illuminated one. In this way the photoresponse for each larval age was measured in each of 3 boxes. After 30 min the number of larvae in each visual environment was recorded. The photopositive line was formed at 24 and 72 h of larval development by selecting in each generation 15 individuals of 24 and 72 h of age found in the illuminated area. Each set of 15 larvae of 24 and 72 h of development was formed by taking ar random 5 larvae from each of the corresponding set of 3 boxes. They were transferred to vials filled with medium, and the adults emerging from them produced the next generation. Similarly, the photonegative line was obtained by selecting two groups of 15 individuals at 24 and 72 h of larval development from the dark area of the boxes from which the photopositive lines were formed. Mortality was also measured by counting the number of adults emerging from vials in which the groups of 15 larvae were placed. Sex ratio was not observed.

Individual development of larval photoresponse

For another comparison of the original population (the Oregon R-C strain) with the lines selected at 24 h, the proportion of photopositive and photonegative larvae was examined in the S_4 generation of selection between 24 and 96 h of larval age. Following hatching, the photoresponse of 60 larvae from the selected lines and the original population were scored individually in each 5 min interval up to 30. Each larva was reared individually on a spoon filled with medium until 48 h when its response to light was again measured. Individual photoresponse was also scored at 72 and 96 h of development. Thus, successive records between 24 and 96 h were obtained for every larva. About 5% of larvae died before 96 h, presumably due to handling. These individuals were replaced by others treated and tested in the same way as those in which development proceeded normally. Larvae reared individually did not show appreciable differences in developmental time compared with those reared in groups (for

other details about this method see Godoy-Herrera *et al.* 1984).

The larvae were sorted into 3 kinds of phenotypes: a) photonegative larvae (larvae (−), those that in 6 repeat trials were found 4 or more times on the dark zone, b) photopositive larvae (larvae (+), those that of the 6 repeat trials performed were observed at least 4 times in the illuminated area of the box, and c) undecided larvae (larvae ±), those that were found in exactly 3 trials in the illuminated zone of the box. Photonegative, photopositive and undecided larvae were again tested three times in the boxes. We wanted to be sure that our classifications 4 out of 6 and 3 out of 6 trials were reliable to discriminate between the three photophenotypes here described. About 8% the larvae tested showed an erratic photophenotype. These larvae were left out of our calculations.

Genetics of larval photoresponse

A further set of experiments was carried out to investigate the hereditary basis of photoresponse of *D. melanogaster* in the larvae at 24 h of age. Files of the S_4 generation of the lines selected at 24 h were used. In this generation our selection scheme was most effective. Again, the individual photoresponse of 60 larvae for each of the selected lines was scored. A group of 20 larvae (−) from the 60 measured in the photonegative line was transferred to vials and reared until emergence. Similarly, a group of 20 larvae (+) from the photopositive line was also reared until adults. These two groups were reciprocally crossed to obtain the generation F_1, and the photoresponse of a group of 60 F_1 larvae of 24 h was measured. After that we took at random a sample of 20 F_1 adults from the group of 60 F_1 individuals scored. They were crossed and the photoresponse of 60 F_2 larvae of 24 h was again measured individually.

Before employing a two-way analysis of variance (ANOVA) and a *t* test for the significance of differences between percentages (Sokal & Rohlf 1969), and arcsine scale was used to transform data.

RESULTS

Larval dispersal and photoresponse

Fig. 1 shows the percentage of larvae of 24, 48, 72 and 96 h age of the Oregon R-C strain in the dark area of the boxes within an observation period of 1 h. Larvae were deposited between the dark and light areas. The percentage of larvae in the dark decreases with larval age. At 24 h of development about 60% of larvae are found in the less illuminated area, but only 10% at 96 h of age. Fig. 1 also shows that the percentage of larvae of first (24-28 h) instar in the less illuminated area does not substantially change during a period of 1 h (two-way ANOVA. $F_{(5, 48)}$ for differences between times = 1.93, $P > 0.05$; $F_{(3, 48)}$ for differences between ages = 3,005.15, $P < 0.01$; $F_{(15, 48)}$ for interaction times x ages = 3.25, $P < 0.001$).

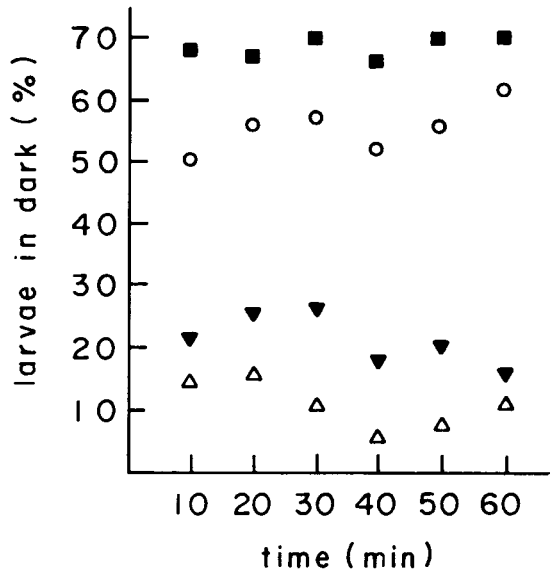

Fig. 1: Percentage of larvae in the dark at 24 (■), 48 (○), 72 (▲) and 96 (△) hours of development (The Oregon R-C strain). The observation period was for 1 hour. Larvae tested were deposited on the limit between the more illuminated area and the dark zone of the boxes (untransformed data).
Porcentaje de larvas en oscuridad a las 24 (■), 48 (○), 72 (▲) y 96 (△) horas de desarrollo (cepa Oregon R-C). El período de observación fue de una hora. Las larvas probadas se depositaron en el límite entre el área más iluminada y el área oscura de las cajas (datos no transformados).

Because time is not significant we used an *a posteriori* test (the Scheffé-method) to determine the significance of differences between each of the 4 age-classes. We found that differences among the means of larvae in dark at 24 and 72 h of larval age were greater than the critical quantity F' = 10.47 (df_1 = 3; df_2 = 20). Significance of difference between 72 and 96 h of larval development was, however, not statistically important (F' = 8.13; df_1 = 3; df_2 = 20). We conclude, therefore, that larval photoresponse of *D. melanogaster* changes significantly between 24 and 72 h of larval development.

When Oregon R-C larvae were deposited either in the dark area or in the light area of the boxes similar results to those illustrated in Fig. 1 were obtained. Constancy in larval photoresponse for a period of 1 h was also obtained in the case of *vestigial* and *yellow* larvae of first, second, early third and late third instar. Fig. 2 shows percentages in the dark of Oregon R-C, *vestigial*, and *yellow* larvae at 24, 48, 72 and 96 h of development. The means were obtained from 6 controls done every 10 min (see Methods). As larval development proceeds, the percentage of larvae in the dark area tends to decrease, with the exception of the *yellow* strain which at 96 h of larval age shows a greater percentage of larvae in the dark area than at 72 h (Fig. 2(c, d)). It is interesting to note that we obtained the lowest percentage of *yellow* larvae in dark (38.92%) at 72 h of larval age.

The Oregon R-C, *vestigial* and *yellow* strains differ principally in the percentage of larvae in the dark area at 24 and 96 h. An *a posteriori* test (the Scheffé method) showed that differences between the means of larvae in dark at 24, 48, 72 and 96 h of the Oregon R-C, *yellow* and *vestigial* strains were greater than the critical quantity F' = 7.36 (df = 2; df_2 = 15); however, the means of larvae in dark at 48 h of the Oregon R-C and *vestigial* strains did not differ between them (F' = 5.62). These findings again suggest that differences in larval photoresponse between the Oregon R-C, *vestigial* and *yellow* strains are age-related.

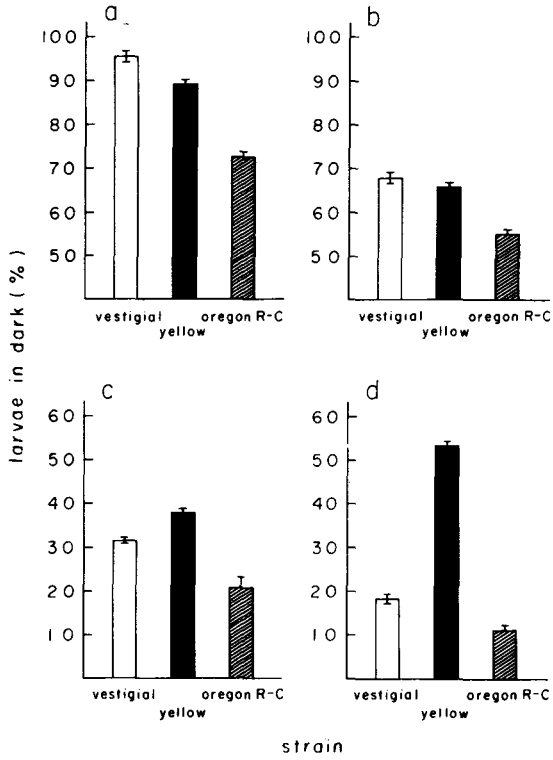

Fig. 2: Percentage of larvae in the dark in the Oregon R-C *vestigial* and *yellow* strains at 24 (a), 48 (b), 72 (c) and 96 (d) hours of development. Larvae were initially placed on the edge between the more illuminated area and the dark area of the boxes (untransformed data).

Porcentaje de larvas en la oscuridad en las cepas Oregon R-C, *vestigial* y *yellow* a las 24 (a), 48 (b), 72 (c) y 96 (d) horas de desarrollo. Las larvas inicialmente se dispusieron en el límite entre las áreas más iluminadas y menos iluminadas de las cajas (datos no transformados).

Genetic selection for the development of larval photoresponse

The results of the selection experiments are summarized in Fig. 3 (a, b). It can be seen that the percentages of larvae in the dark area at 24 and 72 h in the original population (the Oregon R-C strain) were respectively 70 and 20%. During 10 generations of selective breeding it was not possible to modify the proportion of larvae of 24 and 72 h old in the dark area. However, this impression is not altogether correct (see Fig. 3). At 24 h, the selection response in the photopositive line is rapid between the S_1 and S_6 generations. By the S_3 and S_4 generations of that line the means of

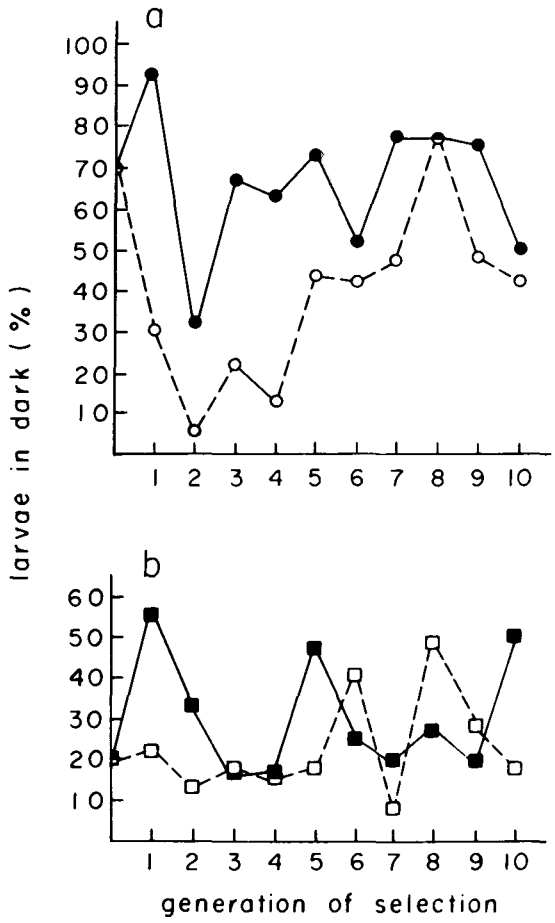

Fig. 3: Selection for positive and negative photoresponse in *D. melanogaster* larvae at 24 (a) and 72 (b) hours of development. Ordinate, the photoresponse score expressed as the percentage of larvae in the dark; abcissa, generations of selection. Black symbols, negative selection; white symbols, positive selection (untransformed data).
Selección para fotorrespuesta positiva y negativa de las larvas de *D. melanogaster* a las 24 (a) y 72 (b) horas de desarrollo. Ordenada, fotorrespuesta expresada como el porcentaje de larvas en la oscuridad; abcisa, generaciones de selección. Símbolos negros, selección negativa; símbolos blancos, selección positiva (datos no transformados).

larvae in the dark area were between 21 and 13%; in the S_5 and S_6 about 41% of larvae were found in the dark area. The selection response in the photopositive line (24 h larvae) continued to overlap with the photonegative line (the S_7 to S_{10} generations). Thus, the differences between the two lines selected at 24 h decrease drastically later being equal at the S_8 generation.

The means of morlality in the selected lines are shown in Table I. In the four lines, mortality in the later generations (S_6 to S_{10}) is greater than the earlier generations. The absolute mortality is greater for selection at 24 h than for selection at 72 h, even though the latter has higher mortality indices. The indices of mortality for the S_6 to S_{10} generations of the photopositive and photonegative lines selected at 72 h were respectively 2.02 and 2.54. In contrast, the indices for the S_1 to S_5 generations of these same lines fluctuate between 1.12 (the photonegative line) and 1.59 (the photopositive line). This situation changes when the mortality indices for the lines selected at 24 h of larval development are examined. In this case the mortality indices fluctuates between 0.97 (S_1 to S_5 generations of the photopositive line) and 1.43 (S_6 to S_5 generations of the photonegative line). A *t* test for the significance of differences between percentages (arcsine transformation) showed the following values: i) 24 h, 1.35 (photopositive line) and 0.65 (photonegative line); $p > 0.05$, df = 1), ii) 72 h, 1.07 (photopositive line), $p > 0.05$, df = 1) and 7.24 (photonegative line), $p < 0.05$, df = 1).

For another comparison of the original population with the lines selected at 24 h of larval age, the proportions of photopositive (+), photonegative (−) and undecided (±) larvae were ascertained in the S_4 generation at 24, 48, 72 and 96 h of larval age. Succesive individual scores were made between 24 to 96 h for a group of 60 larvae. The results are shown in Fig. 4 (a, b, c, d). As expected, at 24 h the photonegative line shows a smaller proportion of (+) larvae than the base population and the photopositive line (*t* values (arcsine transformation) were 4.55 and 6.22; P. two tailed < 0.01) (Fig. 4a). This last line differs from the original population in the proportion of (+), (−) and (±) larvae computed at 48 h of larval development (*t* values were 7.80, 7.33 and 3.95; P. two tailed < 0.01) (Fig. 4b). The larval photophenotypes (−) and undecided (±) are not found at 72 and 96 h of development in the photo-

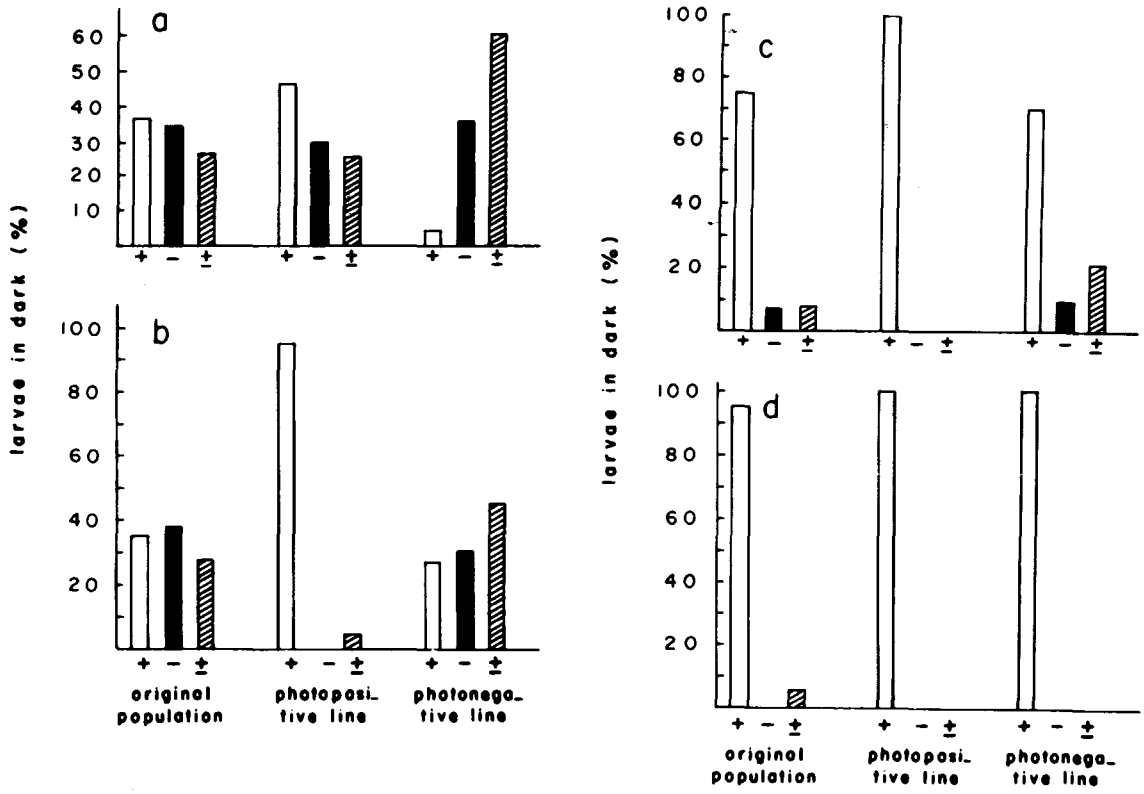

Fig. 4: Percentage of photopositive (+), photonegative (−) and undecided (±) larvae in the base population and the photopositive and photonegative lines selected at 24 hours of larval development; (a) larvae 24 hours; (b) larvae 48 hours; (c) larvae 72 hours; (d) larvae 96 hours. Photopositive larvae are those individuals that were found in the more illuminated area in at least 4 out of 6 repeat trials. Undecided larvae, those individuals found in exactly 3 out of 6 trials in the more illuminated area (see also Materials and Methods; untransformed data).

Porcentaje de larvas con una respuesta a la luz positiva (+), negativa (−) e indecisa (±), en la población base y en las líneas fotopositiva y fotonegativa seleccionadas a las 24 horas de desarrollo larval; (a) larvas de 24 horas; (b) larvas de 48 horas; (c) larvas de 72 horas; (d) larvas de 96 horas. Las larvas fotopositivas corresponden a individuos encontrados en el área más iluminada en al menos 4 de 6 controles sucesivos. Las larvas indecisas corresponden a aquellos individuos registrados 3 de 6 controles sucesivos en la zona más iluminada (ver Materiales y Métodos; datos no transformados).

positive line, increasing the proportion of (+) larvae to 100% (Fig. 4c, d). In contrast, the distribution of larval photophenotypes (+; −; ±) at developmental age of 48 and 72 h in the photonegative line is similar to that of the base population (Fig. 4b, c). At 96 h, only the photonegative line differs from the original population in the proportion of (+) larvae (t = 2.47; P. two tailed < 0.05). These results again suggest that the selection pressure applied at 24 h of larval age substantially affected only the photoresponse of larvae of 24 and 48 h old.

Genetic analysis of selected lines

The distribution of photopositive (+), photonegative (−), and undecid (±) larvae for the lines selected at 24 h of larval development (S_4 generation of selection where we found greatest difference) and for the F_1 and F_2 generations obtained from these two lines are shown in Fig. 5 (a, b, c). Backcrosses were not made. The F_1 shows a phenotypic distribution of the three phenotypes similar to that of the photopositive line (t values were non-significant). By contrast, the F_1 dif-

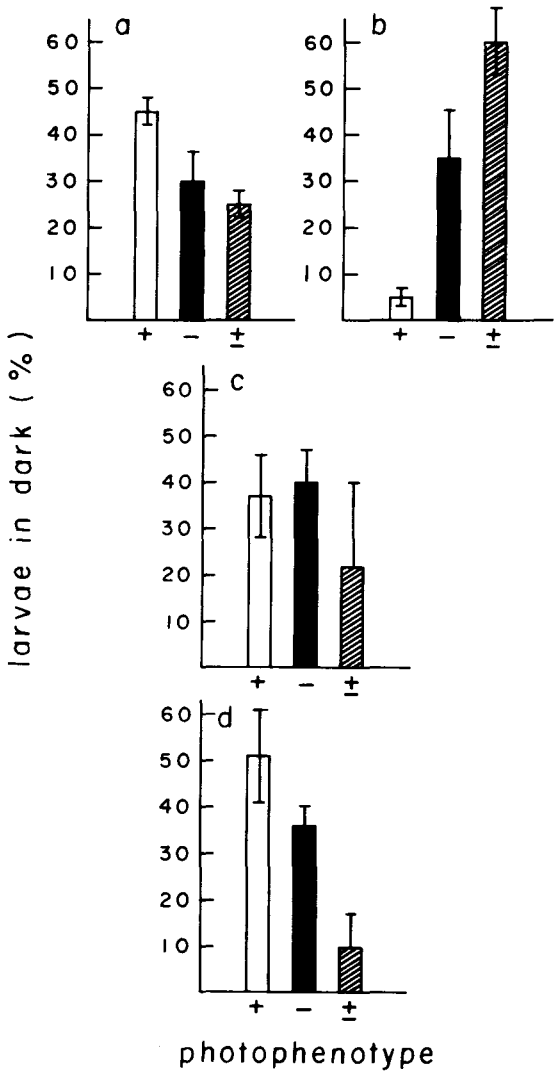

Fig 5: Distribution of larval photophenotypes in hybrid larvae at 24 hours of age obtained by crossing adult flies of the photopositive (a) and photonegative (b) lines; (c) F_1 hybrid larvae; (d) F_2 hybrid larvae. The flies crossed were of the S_4 generation of selection (see Figure 3a). Symbols (+), (−) and (±) as in Figure 4 (untransformed data).

Distribución de fotofenotipos larvales en larvas híbridas de 24 horas de edad obtenidas al cruzar adultos de las líneas fotopositiva (a) y fotonegativa (b); (c) larvas híbridas F_1; (d) larvas híbridas F_2. Las moscas cruzadas eran de la generación S_4 de selección (ver Figura 3a). Símbolos (+), (−) y (±) como en la Figura 4 (datos no transformados).

fers from the photonegative line in the means of (+) and (±) larvae (*t* values were respectively 4.36 and 5.64; P. two tailed < 0.05). As would be expected, the F_2 generation shows a phenotypic variation greater than the F_1 (the F_1 variance employing an arcsin transformation was 7.96, while that of the F_2 was 11.31; $F = 1.42$; P. two tailed < 0.05, $df_1 = df_2 = 59$). These results suggest genetic segregation for larval photoresponse at 24 h of larval age.

DISCUSSION

The present work shows that the responses of *D. melanogaster* larvae to light varies with genetic background and developmental stage. Larvae of the Oregon R-C, *vestigial* and *yellow* strains show substantial differences in their photoresponsiveness at 24 and 96 h of age. However, 48 and 72 h old larvae of these same strains show similar photobehavior. Thus, phenotypic variance for larval photoresponse associated with genetic differences is age-related. *D. melanogaster* larvae at 24 h of age exhibit many activities on the surface of the culture medium. They search for any scars or holes in the surface of the medium where they can gain entrance into the medium in order to burrow. The 96 h old larvae (late third-instar larvae) show a characteristic pre-pupation behavior (Godoy-Herrera *et al.* 1984, Sokolowski *et al.* 1986). In contrast, 48 and 72 h old larvae of *D. melanogaster* show a propensity to dig into the culture medium (Godoy-Herrera 1986). Changes in the development of larval photoresponse of *D. melanogaster* could be associated with the different light environments inhabited by the larva at different points in its life.

On the other hand, two-way ANOVA showed that the differences in the percentages of larvae at a given age in the dark over time were not significant, suggesting that the behavioral response measured was essentially the larval photoresponse and not, for instance, any larval thermal response. In this last case, we sould have registered significant variations in the number of larvae in the dark as time goes by because the temperature in the boxes would be increasing (see also Cáceres & Vega 1983).

The response to selection for larval photobehavior is also age-related. At 24 h we obtained a response to selection for this behavior in only the photopositive line, but at 72 h it was not possible to detect a response to selection for this character. These findings suggest that perhaps the magnitude of genetic homeostasis changes with larval development and this might affect the heritability of this trait. This could explain why we were unable to obtain any discernible selection response at 72 h. In connection with these suggestions, it is interesting to examine larval/adult mortality. At 72 h of larval development, mortality is proportionally higher than at 24 h. This is in good agreement with the idea that genetic homeostasis is changing with larval development and that it seems to be greater at 72 than 24 h developmental age. If heritability decreases with larval age as a consequence of changes in genetic homeostasis, then it should become increasingly difficult to select for differing larval photoresponse. In fact, phenotypic variance between the Oregon R-C, *vestigial* and *yellow* strains at 72 h of larval development is lower than at 24 h of larval development. Little is understood about the relationship between genetic homeostasis and heritability. Perhaps the genes involved in the control of larval photophenotypes of *D. melanogaster* are part of an equilibrated system controlled by natural selection, similar to those proposed by Mather & Harrison (1949) for chaeta number and fertility in *Drosophila* an by Lerner (1970) for shank length and viability in chickens.

Additional evidence for genetic homeostasis comes from the response to selection for larval photoresponsiveness at 24 h. The response in the photopositive line was rapid between S_1 and S_4 generations, but between S_5 and S_{10} the line tended to overlap with the photonegative line, suggesting that perhaps the integrated gene system resists change under the selection pressure introduced. The tendency of a population to balance its genetic compositions resisting sudden changes is related to the particular level of heterozygosity (Lerner 1970, Livshits & Kobyliansky 1985). Even if we do not have sufficient evidence to support the idea that homozygosity produced by directional selection applied at 24 h of larval development reduced the fitness, it is interesting to observe that mortality in the selected lines was 33 to 43% higher than in the base population. This seems to suggest that the coadapted gene complexes in which genes for photoresponse could be involved requires a certain level of heterozygosity.

Ricker & Hirsch (1985) succeeded in breaking the homeostasis of genes, producing a new balance of equilibrium after 550 generations of selection for negative and positive geotaxis in adult populations of *D. melanogaster*. Consequently, when selection was relaxed the populations maintained their response. These authors also showed that if one reverses selection, the mean returns toward the extreme. This also argues for a reversal in genetic homeostasis. The contrast between our results and those of Ricker & Hirsch could be explained if we think that we selected for only 10 generations whereas Ricker & Hirsch reported over 500 generations, enough time to achieve large changes in coadaptation. *Drosophila* like other holometabolous insects, feed and grow during the larval stage, while adult flies are concerned primarly with reproduction. Consequently, in comparison with those of adults, larval behavior patterns are probably subject to different selection pressures. This means that behavior patterns of larvae and adults of *D. melanogaster*, which serve very different functions, should evolve at different rates.

The results of this research indicate that selection applied at 24 h affects the photobehavior of first and second instar larvae. At 72 and 96 h, photophenotypic distributions of the selected lines are similar to those of the base population. These findings suggest a switch in the hereditary program controlling larval photoresponse of *D. melanogaster;* the switch seems to occur between 48 and 72 h of larval ages. This photobehavioral difference between larvae of the first and second instars and those of the third instar seems

to reflect and epigenetic divergence in the architecture of genotypes controlling this behavior. Godoy-Herrera *et al.* (1984) reported that young larvae (24, 48 and 72 h of development) of *D. melanogaster* show a preference for moist substrates, but mature larvae (96 h of age) prefer frier substrates. This last behavior is linked with choice of pupation site. Thus, during the larval stage of *D. melanogaster* a genetically programmed sequence of changes in a variety of larval behavioral patterns seems to occur (see also Sokolowski *et al.* 1984; Troncoso *et al.* 1987). These behavioral changes might contribute to the adaptation of *D. melanogaster* larvae to the different habitats occupied by these individuals at different periods of their life.

The crosses between the lines selected at 24 h show departures from additivity. In fact, they suggest some dominance for photopositive response. More extensive genetic analysis should be undertaken to define more precisely the genetic control of larval photoresponse of *D. melanogaster* at different larval ages. For instance, we do not know whether undecided larvae are within the range of potential phenotypes that a (+) genotype could develop if exposed to a specified range of environmental conditions. Probably the larval photoresponse of *D. melanogaster* may lead larvae at each instar to develop in specific microhabitats, restricting, for example, young larvae (24 and 48 h of age) to feed in microenvironments qualitatively different from those exploited by older larvae (72 h).

A variety of behavioral factors are involved in habitat choice in *Drosophila* (Parsons 1983). The present work indicates that *D. melanogaster* larvae of different ages may use their photoresponse to influence the direction of their movements. Differences in photoresponse between larvae of various ages are related to epigenetic changes in the genetic architecture of this behavior.

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