

Table 1. Principal hydrocarbons of *hs-tra* males after various heat-shock treatments. Data shown are the mean ($\pm$ se) of the principal hydrocarbons given in nanograms. 7-T = 7-tricosene; 7-P = 7-pentacosene; 7,11 HD = 7,11 heptacosadiene; 7,11 ND = 7,11 nonacosadiene. The sum of all detected hydrocarbons (sum Hcs) is also provided.

Hydrocarbons	7-T	7-P	7,11 HD	7, 11 ND	Sum Hcs
treatment 0	303 $\pm$ 30	322 $\pm$ 19	34 $\pm$ 3	0	1211 $\pm$ 74
treatment 1	250 $\pm$ 33	380 $\pm$ 35	195 $\pm$ 39	45 $\pm$ 11	1753 $\pm$ 84
treatment 2	70 $\pm$ 14	204 $\pm$ 36	179 $\pm$ 36	52 $\pm$ 8	1335 $\pm$ 146
treatment 3	26 $\pm$ 3	100 $\pm$ 10	117 $\pm$ 38	60 $\pm$ 12	990 $\pm$ 142
treatment 4	9 $\pm$ 2	50 $\pm$ 6	28 $\pm$ 4	8 $\pm$ 3	509 $\pm$ 85

treatment 0: no heat-shock; treatment 1: dry incubator, glass vial (18cc) with food (4cc); treatment 2: dry incubator, plastic vial (11cc) without food; treatment 3: water-bath incubator, plastic vial (11cc) without food; treatment 4: water-bath incubator, plastic vial (11cc) with food (4cc).

Table 2. Principal hydrocarbons of *hs-tra* females after various heat-shock treatments. Data shown are the mean ($\pm$ se) of the principal hydrocarbons given in nanograms. For abbreviations and treatments, see Table 1.

Hydrocarbons	7-T	7-P	7,11 HD	7, 11 ND	Sum Hcs
treatment 0	30 $\pm$ 3	82 $\pm$ 4	455 $\pm$ 18	268 $\pm$ 15	1787 $\pm$ 46
treatment 1	39 $\pm$ 2	123 $\pm$ 6	597 $\pm$ 31	376 $\pm$ 20	2587 $\pm$ 93
treatment 2	29 $\pm$ 5	114 $\pm$ 23	185 $\pm$ 38	89 $\pm$ 21	1194 $\pm$ 224
treatment 3	0	7 $\pm$ 2	4 $\pm$ 2	2 $\pm$ 1	276 $\pm$ 25
treatment 4	0	0	0	0	185 $\pm$ 15

time, this procedure had no, or very little effect on hydrocarbons.

—When 3 to 9 hour old *hs-tra* males were placed in a water-bath incubator during one hour, their mature hydrocarbons were nearly or completely absent. Males used in this procedure were grouped by 10 in a 10 x 120 mm polypropylene vial without food. When the same heat-shock procedure was applied either earlier or later during imaginal development, a partial feminization of the principal hydrocarbon could occur, without any decrease of the general level of hydrocarbons.

We found that the second treatment, but not the first one, had a very significant effect on the hydrocarbons borne by 4-day-old female flies. In this case, female lacked all their principal hydrocarbons.

Here, we investigated the production of the four principal hydrocarbons (7-T, 7-P, 7,11HD and 7,11ND) in 4-day-old *hs-tra* males and females treated with different combinations of the parameters 2 and 4 (type of incubator and type of vial) described above. All the flies were 3-hour-old adult when treated, and the heat-shock lasted one hour.

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Two different treatments combining these parameters induced very extreme phenotypes with regard to the principal hydrocarbons in 4-day-old *hs-tra* males:

—When 12 to 48 hour-old *hs-tra* males were placed in a dry incubator during two hours, their mature hydrocarbons were largely or completely feminized. In this case, males were grouped by 10, in a glass vial (18 cc) containing 4 cc of standard corn food. If the heat-shock was applied before or after that critical period of



Behavioral interactions in the sibling species *D. pavani* and *D. gaucha* in stressing environments.

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Environmental changes that originated responses above individual umbral are called stressing factors (Chrousos and Gold, 1992; Hoffman and Parsons, 1994). Stress may be measured in terms of behavioral and/or somatic changes (Beerda *et al.*, 1999a, 1999b; Djawdan *et al.*, 1998). In particular, behavioral modifications caused by stressing factors could reflect changes at the central nervous system level. These behavioral changes should improve the individual capacity to adjust to stressing environments (Spruijt *et al.*, 1992; Chrousos and Gold, 1992). On the other hand, whether a particular environmental condition is or is not stressing depends on genetic endowment of each individual (Zhivotovsky, 1997). Thus, it is important to investigate behavioral changes in relation to specific stressing environmental factors and individual genotypes or groups of genotypes. *Drosophila* species are a good model to undertake this type of investigation. For example, crosses between *Drosophila* species may produce abundant hybrids that survive to adulthood. These hybrids could offer an opportunity to investigate the role of genotype in the behavioral responses to stressing environments. Also, investigations on behavior and stress by using closely related *Drosophila* species could be of importance to understand better their distribution patterns in the wild.

Here we report on some behavioral changes in adults of the sibling species *D. pavani* (endemic in Chile) and *D. gaucha* (endemic in Argentina and Brasil) to be transferred from their rearing environment to stressing environments. The individuals used were originated from adults collected in Chillán (*D. pavani*), 400 km south of Santiago (Chile) and in Buenos Aires (*D. gaucha*) (Argentina). After hatching, females and males of 2 - 4 h of age were individually deposited in vials with food which had available 36 cc of free space. The vials were maintained under constant illumination at 24°C. After 10 - 12 days, each one of the flies was transferred into a new vial with food and the behavior of each individual was observed. These were the controls for the next experiments. Other groups of males and females of the two species of the same age were individually introduced in vials with food with 18 cc of free space. Again, the behavior of each individual was recorded. In a third experiment, females and males of *D. pavani* and *D. gaucha* were individually distributed in vials without food and their behavior was observed. In each one of the experiments the behavior of 30 individuals of each sex and species was recorded for 2 min. The behaviors recorded were: i) locomotion (l), moving from one place to another, ii) turning (t), changes in direction when moving, iii) jumping (j), flies jump from one place to another, iv) still (s), stopping behavior, v) grooming (g), cleaning legs and/or body, vi) falling down, flies fall on their back, vii) flight, fly in air, the last two behaviors were exhibited at a very low frequency. Thus, they were grouped under the term "other" (o). The collected data were used to build ethograms showing interactions between the behaviors. The ethograms were compared to each other by using the Mantel's test (Sokal and Rohlf, 1995).

Figure 1 shows the results in rearing vials (controls), vials with food and reduced space, and vials without food. In the rearing environment (controls) the two sexes of *D. pavani* and *D. gaucha* exhibit transitions locomotion ↔ turning, and locomotion → still. In addition, *gaucha* males show transitions locomotion ↔ jumping. In vials with reduced space, again adults of *D. pavani* and *D. gaucha* females show transitions locomotion ↔ turning, locomotion → still. On the other hand, *gaucha* males exhibit only transitions locomotion ↔ turning. In the vials without food, males and females of the two species show transitions locomotion ↔ turning, *gaucha* males also show transitions grooming ↔ still and recurrence for grooming.

The Mantel's test yielded the following results when the ethograms (Figure 1) were compared: i) *pavani* males, controls vs reduced space plus food, $r = 0.76$; controls vs vials without food, $r = 0.86$, ii) *pavani* females, controls vs reduced space plus food, $r = 0.91$; controls vs vials without food, $r = 0.61$, iii) *gaucha* males, controls vs reduced space plus food, $r = 0.88$; controls vs

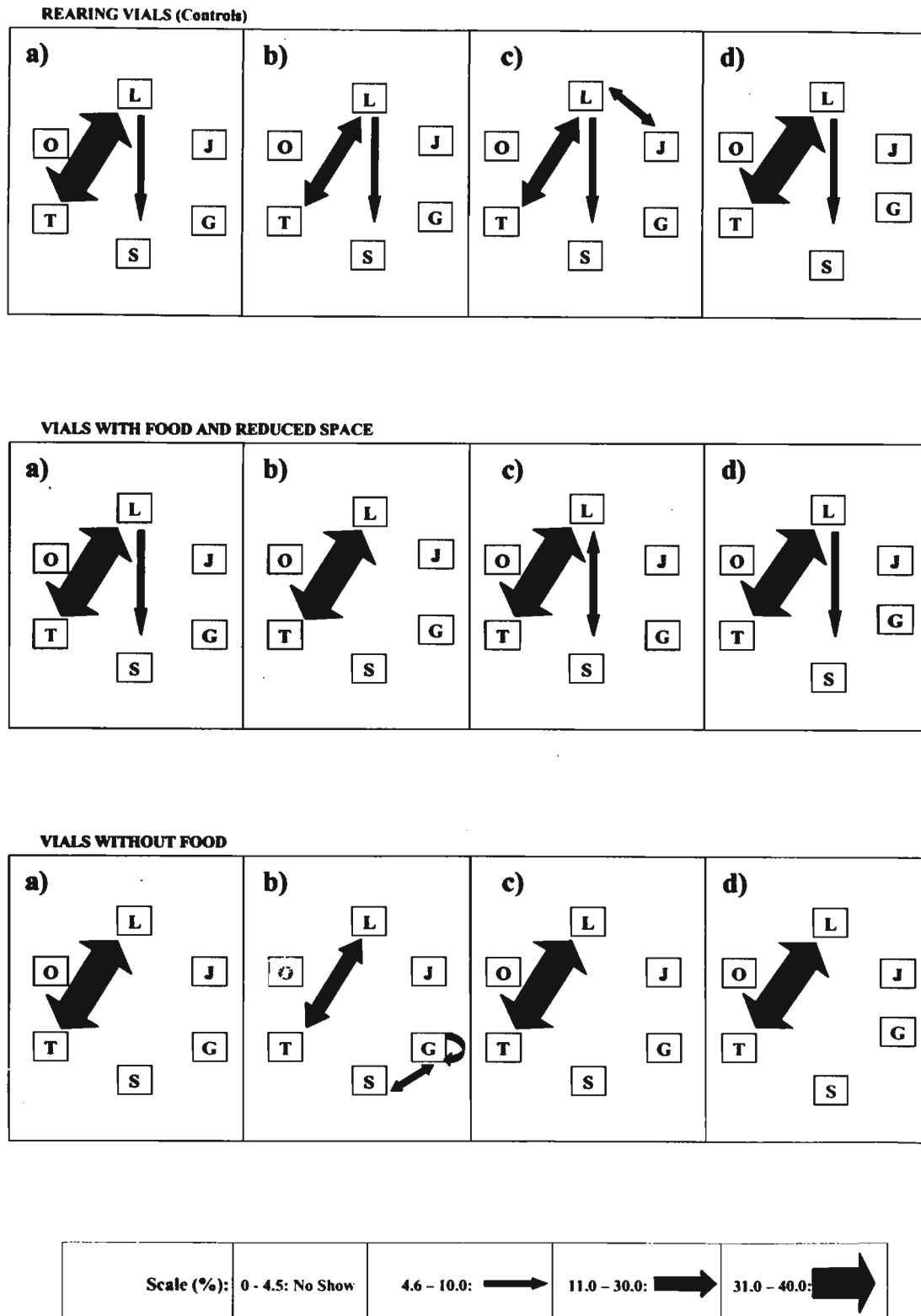


Figure 1. Ethograms showing relationships between behaviors of males and females of *D. pavani* and *D. gaucha*: (a) *pavani* males, (b) *gaucha* male, (c) *pavani* female, (d) *gaucha* female, in: i) vials with food, a normal environment (controls), ii) vials with reduced space, and iii) vials without food and water.

vials without food, $r = 0.58$, iv) *gaucha* females, controls vs reduced space plus food, $r = 0.89$; controls vs vials without food, $r = 0.69$. Correlation (r) coefficients under 0.7 indicate very poor fit between the pairs of matrices which are compared; r between 0.7 and 0.8 indicates a poor fit; r between 0.8 and 0.9 indicates a good fit; and r equal or over 0.9 indicates a very good fit between the matrices (Rolhf, 1995). Thus, the results of the Mantel's test above obtained a good agreement with the ethograms (Figure 1).

In the absence of food, adult flies of *D. pavani* and *D. gaucha* change their behaviors, as shown by new transitions that occur between the behavioral elements recorded, while other behaviors exhibited in the presence of food disappear. We also found that in the same environment, adults of *D. pavani* may react differently than *D. gaucha* adult flies. This is in agreement with Clark (1998) in the sense that different genetic systems may respond in different ways to the same environmental changes. However, only changes in grooming behavior reflect stress (Spruijt *et al.*, 1992; Eguibar *et al.*, 1997; Lawer and Cohen, 1988). Grooming represents a nervous activity behavior that is variably expressed when "located" among a well known environment or a stressing one. Changes in transitions locomotion $\leftrightarrow$ still could merely represent adjustment of each sex of the sibling species to a new environment which is not necessarily stressing (Beerda *et al.*, 1999; Clark *et al.*, 1997). Our findings indicate that only males of *D. gaucha* exhibited recurrence and transitions for grooming behavior. Thus, this sex of *D. gaucha* could be representative indicators of stress when they are transferred to an environment where food and water are not available.

References: Beerda, B., M.B. Schilder, J.A. van Hooff, H.W. de Vries, and J.A. Mol 1999, *Physiol. Behav.* 66: a) 233-242, b) 243-254; Chrousos, G.P., and P.W. Gold 1992, *JAMA* 267:1244-1252; Clark, A.G., and C.D. Fucito 1998, *Heredity* 81:514-527; Clark, J.D., D.R. Rager, and J.P. Calpin 1997, *Lab Anim. Sci.* 47:571-579; Djawdan, M., A.K. Chippindale, M.R. Rose, and T.J. Bradley 1998, *Physiol. Zool.* 71:584-594; Eguibar, J.R., and A. Moyaho 1997, *Pharmacol. Biochem. Behav.* 58:317-322; Hoffmann, A.A., and P.A. Parsons 1994, In: *Evolutionary Genetics and Environmental Stress*, Oxford University Press Inc. New York, U.S.; Lawer, C.P., and P.S. Cohen 1988, *Ann. NY Acad. Sci.* 525:417-419; Rolhf FJ NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Version 1.80. State University of New York, Stony Brook, NY. Exeter Software; Sokal, R.R., and F.J. Rolhf 1995, In: *Biometry*, second edition, Freeman & Company, New York; Spruijt, B.M., J.A. van Hooff, W.H. Gispen 1992, *Physiol. Rew.* 72:825-852; Zhivotovsky, L.A., 1997, *EXS* 83:241-254.



A case of direct measurement of coefficients of selection in nature.

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The mathematical theory of selection developed by Fisher, Haldane and Wright assumes that coefficients of selection are constant. The usual result thereof, in the absence of heterozygotes' advantage, is elimination and fixation of alleles. The latter is considered as an elementary act, one of those which result in speciation that is considered as transmutation (Timofeev-Resovsky *et al.*, 1973). In contrast to this, observations of many researchers have resulted in establishing that 1) there is no allele fixation in populations, and polymorphism is conserved in each locus; 2) the polymorphism is