The contents of E and 20HE in females of lines 101 and 147 (Figure 1) under normal conditions demonstrate that there are differences between lines 101 and 147 in levels of the ecdysteroids. In mutant (line 147) females, both compounds are considerably higher than in wild type ones (differences are significant at $P < 0.001$ for E, and $P < 0.01$ for 20HE).

It is also clear that in wild type females (line 101) under stress both ecdysteroids increase (differences from the control are significant at $P < 0.01$ for 20HE and $P < 0.001$ for E). In mutant females under stress, the levels of E and 20HE do not change. Thus, the system of ecdysteroids is one of the components of stress-reaction in *Drosophila*.

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Heat induced male sterility in *Drosophila buzzatii*: Genetic variation among populations for the duration of sterility.

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Spermatogenesis in *Drosophila* males is blocked at or above 30°C and below 13°C, and causes males to be sterile, but a return to 25°C restores fertility within some days (David *et al.*, 1983). The narrow temperature interval that constitutes the limit between fertility and sterility is apparently fixed at a very specific temperature (David *et al.*, 1983). Attempts to push the threshold for temperature-induced sterility by artificial selection have not been successful (David pers. comm.).

When temperatures that cause male sterility are increased further, the duration of sterility is prolonged (David pers. comm.). This correlation between the stressfulness of the environment and the duration of sterility may imply that the latter can be genetically variable because thermal adaptation is common in *Drosophila* and because different populations experience high temperatures as more or less stressful.

The aim of the present study was to examine if genetic variation for the duration of heat induced sterility is present among populations despite a lack of variation in the temperature threshold for heat induced sterility. Three different populations of *Drosophila buzzatii* from Catamarca and Tilcara in Argentina and Tenerife in Spain were used. Development from larvae to adult was allowed at two different thermal regimes: 25°C and 31°C.

Seven days-old flies from all populations were put in separate bottles for egg laying at 25°C. The flies were transferred to new bottles after 24h to obtain enough flies for the experiment. Two days after the beginning of oviposition, bottles were placed in the two temperature chambers. As flies exposed to different heat treatments did not hatch at the same time, new bottles were set up to ensure

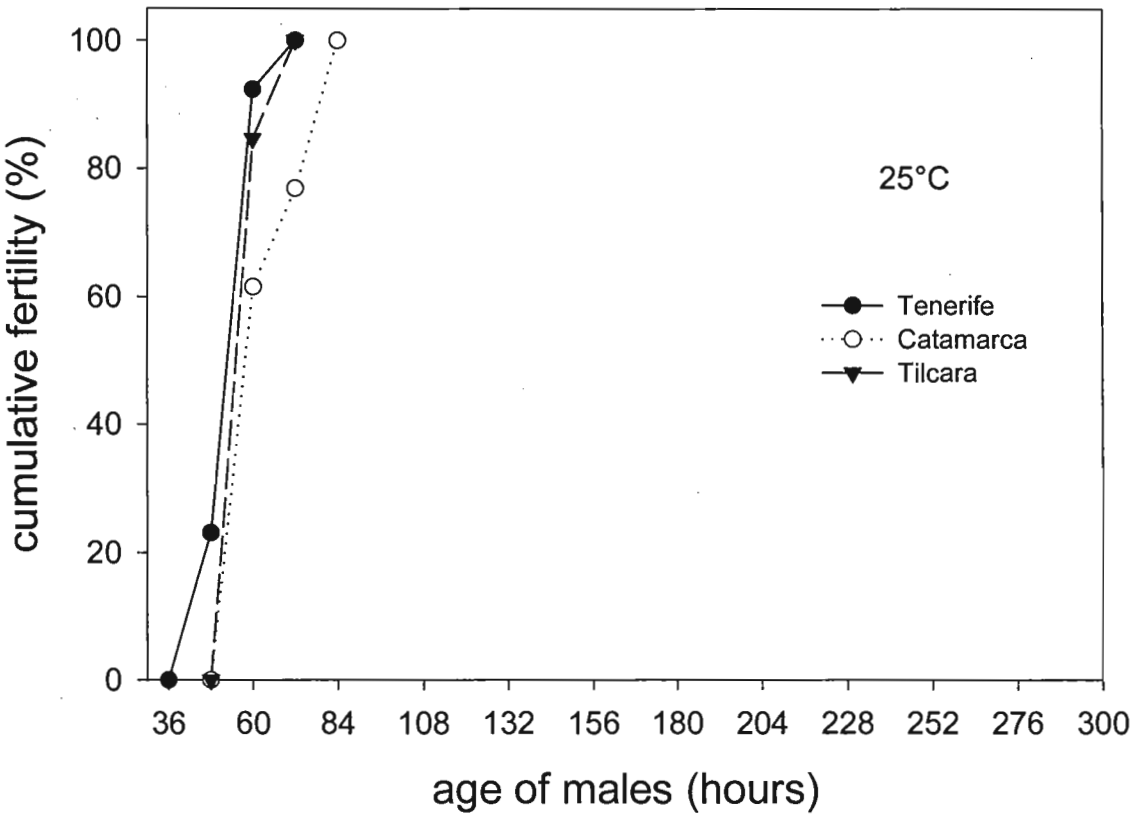

Figure 1. Cumulative percentage of vials with at least one fertile *Drosophila buzzatii* male at each age (hours). Each vial contained four males and four females reared at 25°C in the period from egg to adulthood.

hatching over a longer period. This allowed the duration of sterility to be measured at the same time in all combinations of populations and treatments. Instant *Drosophila* medium (Carolina Biological Supply) with a little yeast added was used during the whole experiment. Newly hatched males (on average 6h old) were placed in vials with six day-old virgin females from their own population, which had been raised at 25°C. For each combination of population and treatment, 13 replicate vials with four males and four females were placed at 25°C. The four males per vial were used to exclude an effect of occasional sterility of up to 40% (Bundgaard and Barker, 2000), which was confirmed also for the present populations in a pilot study at 25°C. Every 12 hours the flies were transferred to new vials. The time until sexual maturity was achieved for at least one of the four males per vial was estimated as the time until the first viable progeny could be detected. The duration of sterility was calculated from the median of the period where the males eclosed to the median of the period where the new eggs were laid. The vials were kept at 25°C for a minimum of seven days before they were evaluated for the presence of pupae, larvae or adults. This measure of heat-induced sterility includes the period of approximately 3 days where males normally are sexually immature, as seen from the 25°C treatment.

The results are presented as the cumulated percentages of vials where fertility was observed (Figures 1 and 2). It can be seen that some line-treatment combinations never reached 100% fertility, meaning that for some of the vials all four males remained sterile. The cause for this observed

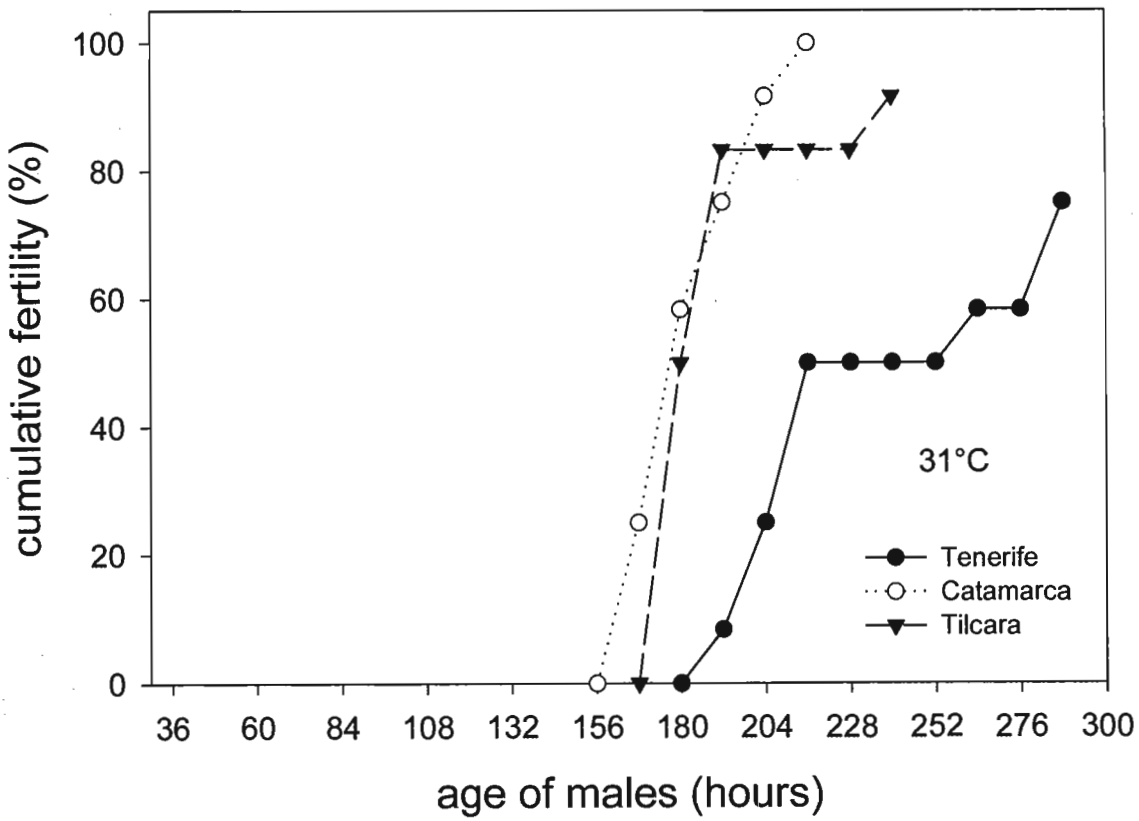

Figure 2. Cumulative percentage of vials with at least one fertile *Drosophila buzzatii* male at each age (hours). Each vial contained four males reared at 31°C in the period from first instar larvae to adulthood and four females reared at 25°C from egg to adulthood.

sterility is not known. Bundgaard and Barker (2000) observed 40% sterility in males in an experiment concerning repetitive mating in *D. buzzatii,* which was performed at 25°C. In the present study vials with sterile males only were found in the heat-treated group, *i.e.* 31°C.

The results show significant variation (analyses not shown) in the time until regained fertility depending on both population (genetic effect) and temperature treatment (environmental effect). At 25°C, all populations are fertile almost at the same time (Table 1 and Figure 1), with the Spanish population being slightly faster. In all vials placed at this temperature fertile eggs could be detected, and, as this temperature is a non-stressful temperature for *Drosophila buzzatii*, males of this species mature sexually in approximately 2.5 days under optimal conditions. At 31°C (Table 1 and Figure 2) the average duration of sterility is longer than at 25°C. At this temperature there is a difference in the duration of sterility of two days between the Argentinean and the Spanish populations with the Argentinean populations being sterile for a shorter period. In only 90% of the vials from Tilcara and in 75% of the vials from Tenerife, offspring could be detected. There is also evidence for an increased variance in the sterility period

Table 1. Age until males were fertile in hours (mean ± SD) of the population from Tenerife (Spain) and the two populations from Catamarca and Tilcara (Argentina).

	Temperature	
Population	25°C	31°C
Tenerife	58 ± 7	225 ± 47
Catamarca	67 ± 10	174 ± 16
Tilcara	62 ± 5	178 ± 18

within populations at 31°C. This increase in variance is most pronounced in the Spanish population where the length of the sterility period was most severely affected by the high temperature stress.

In summary, the results show that the thermal environment affects the duration of heat-induced sterility in *D. buzzatii* and furthermore that genetic variation for this trait is present among populations.

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The synthesis of a double fourth chromosome marked with y^+.

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Here I report the construction of C(4)DRA, a compound of the fourth chromosome that bears two markers, the recessive ci^l and y^+. This chromosome was built by a recombination between C(4)RM, ci^l ey^R and T(1;4)*sc H,* y^+.

I have previously noticed by complementation tests that T(1;4)*sc H,* y^+ is a deficient translocation that deletes at least 11 terminal genes of the fourth (Table 1). Since the fourth chromosome does not recombine normally, to induce recombination between C(4)RM and T(1;4)*sc H*, I made heterozygous animals *y w mit/ y w mit*; C(4)RM/T(1;4)*sc H,* y^+ and heat shocked larvae (1^{st} to 3^{rd}) for 14 hours at 37°C. This treatment was extremely severe and from about 100 larvae, I recovered 1 y^+ adult female. This single female was then crossed to *y w mit*/Y; *spa*[rgo]/*spa*[rugoso-nu] flies to detect the recombination event. *spa*[rugoso-nu] is a new homozygous viable allele of *spa*, that presents *shaven* and *sparkling* mutant phenotypes and is lethal over T(1;4)*sc H,* y^+(see scheme). T(1;4)*sc H,* y^+/*spa*[rgo] produce flies with extensive loss of macrochaeta that die as pupae or soon after hatching.

Any survivor of this cross presenting y^+ and normal macrochaetae may correspond to a recombination between T(1;4)*sc H* and C(4)RM. One female in a progeny of 38 presented was y^+ spa^+ and it was crossed again with *y w mit*/Y ; *spa*[rugoso-nu]/*spa*[rugoso-nu] males. From this cross only emerged animals *y spa*[rugoso-nu] and y^+ spa^+ flies. Some of y^+ spa^+ flies were crossed to the parental chromosome C(4)RM. With this combination I have observed the appearance of ci^l y^+ tetra-4 flies, that indicates a recombination between *ci* and *ey* that replaced ey^R by ey^+. This chromosome was named C(4)DRA-1 (Deleted on the Right Arm) and further tests showed that it is hemizygous viable in males and females, homozygous viable in females but lethal in homozygous males. Since this chromosome is completely viable over C(4)RM and in hemizygous males, I presumed that the lethality observed in homozygous males is due to an excess of a gene located in the X component of this chromosome. This element received the provisory name *male lethal dosage dependent* or *mald.* This chromosome has been quite stable after 6 months of its synthesis, and until now there are no signs that it can become homozygous for ey^R .

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