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INTRAPOPULATION DIFFERENTIATION IN BLACK MUTANTS OF *TRIBOLIUM*

ABSTRACT: The studies were carried out on black mutants distinguished as *Tribolium castaneum* "sooty" and *T. confusum* "ebony-2" both considered as recessive for blackness. The studies aimed at finding the phenotypic differentiation into 6- and 7-instar groups which were actually observed in both the strains, however at different proportions: 1 to 4 in *T. castaneum* "sooty" and 1 to 15 in *T. confusum* "ebony-2". The discerned instar groups differed in length of development and maximum larval weight.

KEY WORDS: *Tribolium*, black mutants, phenotypic differentiation

1. INTRODUCTION

Earlier studies (Prus 1976, Bijok 1986) revealed that genetic strains distinguished as cI *Tribolium castaneum* and bIV *T. confusum* reveal the differentiation of development time into 6- and 7-instar groups. Such differentiation was also found in other strains of *Tribolium* : cIV of *T. castaneum* and bI of *T. confusum* (Prus and Prus 1990). The strains cI – cIV of *T. castaneum* ans bI – bIV of *T. confusum* were described by Park et al. (1961) when studying their primary characteristics. So discerned strains were investigated later in the respect of their primary characteristics such as fecundity and hatchability (Prus and Prus 1987, Prus et al. 1989). Recently they were also tested as to their response to tricalcium phosphate, a salt which is known to suppress the development of pest insects (Prus 1989, 1990, 1995). Strains: *T. castaneum* cI and cIV and *T. confusum* bI and bIV originated from stocks of Professor Park's Laboratory of the University of Chicago (USA) where they are known as genetic strains and were obtained by Park from wild strains *T. confusum* Duval – b "Chicago Standard" and *T. castaneum* Herbst – c "Brasil".(Park et al. 1964)

To check whether other strains of flour beetles,i.e.. black mutants, originated from different sources show phenotypic differentiation similar to that observed in genetic strains is the aim of the present study. Strain "sooty" ("S" for abbreviation) of *T. castaneum* was derived from Professor A. Sokoloff's laboratory of San Bernardino, California. This strain is a black mutant recessive in respect to black colour (Sokoloff 1966, 1972). Another black mutant of *T. confusum*, "ebony-2" (symbol E), originating from the same source, is also recessive for blackness.

2. MATERIAL AND METHODS

The material used for this work was kept in the Professor Klaus Sander's Laboratory in Freiburg, (Germany) where this study was performed. The beetles of the two strains of black mutants have been propagated in adequate numbers and then the experiments were set up. The method used to test the strains for heterogeneity was similar to that applied earlier by Prus (1976) and Bijok (1986) and described elsewhere (Bijok 1989, 1992). It depended on individual culturing of single specimens from newly hatched larvae to the adult stage, each at 1g of culture medium consisting of wheat flour and dry powdered yeast mixed together at weight proportion 20:1. The cultures were placed at random in a dark incubator and run at a temperature of 29°C and relative humidity of 80%.

The census was done daily consisting of checking up the contents of culture medium for the skin cast. Every second day the animals were also weighed on a Sartorius balance to an accuracy of 0.0001g, starting from 7th day. A total of 120 replicates was set up, 60 for each of the two strains: "S" of *T. castaneum* and "E" of *T. confusum*. Some of animals died before eclosion, and their data were not taken into consideration.The results obtained were then drawn as individual growth curves (wet weight changes) of individual grouped according to sex (determined at the pupal stage). The duration of pupal stage was also marked individually on these graphs. Depending on number of exuviae the individuals were classified either as 6- or 7-instar group. Later on, the growth curves were averaged for each instar group separately, resulting in mean growth curves for 6- and 7-instar groups. Frequency of each group within the total number of individuals tested was also determined.

Another experiment performed simultaneously aimed at determining the frequency of appearance of pupae in group cultures of these strains run in defined amount of standard culture medium. To achieve this, synchronized cultures were set up with different densities of newly hatched larvae. In *T. castaneum* "S", density of 177 indiv. · 32 g^{-1}, was considered as low, 380 · 32 g^{-1} as average, and 510 · 32 g^{-1} as high. In *T. confusum* "E" all of densities can be considered as low (140–180 indiv. · 32 g^{-1}). From the moment of appearance of pupae they were removed, counted, weighed in order to assess individual pupal weight and their sex was determined. Then, frequencies of pupae of both sexes appearing in the two strains were drawn against time. It allowed to check whether there was one or two peaks of pupal appearance in a given strain. This would imply the presence of different phenotypic groups in each strain. Thus confirmating the results based on individual cultures. Differences in mean wet weight of pupae against time of their appearance were also tested. If the pupal weight values change from smaller to bigger this will be consider as an indirect proof of existence of the two groups in these populations.

3. RESULTS

Individual culturing of beetles representing black mutants allowed to ascertain that both strains of *T. castaneum* are differentiated into two groups, namely that of 6- and of 7-instar. It can be seen from the course of several wet growth curves (here as an example) (Fig. 1). Males (which had six casts of exuvium during their development) show much smaller maximum weight attained earlier (by several days) and their weight at eclosion is still lower than that of the remaining individuals. These are 6 instar individuals. Other males (which had seven casts of exuvium during their development) had slower development with maximum weight attained later; they achieved maturity later and have higher individual weight. They represent 7-instar group.

Within each sex and instar group the weights were averaged resulting in courses of growth curves for *T. castaneum* "S" and *T. confusum* "E" (Fig 2). One should remember, however, that these averages are burdened with large error due to small number of replicates. In black mutant of *T. castaneum* these groups differred by weight, 6-instars being lighter than 7 instars. The development time in these two groups coincided (Fig. 3). In *T. confusum*, the weight differences were clearly expressed and the maximum weights were observed earlier in 6-instar than in 7-in-

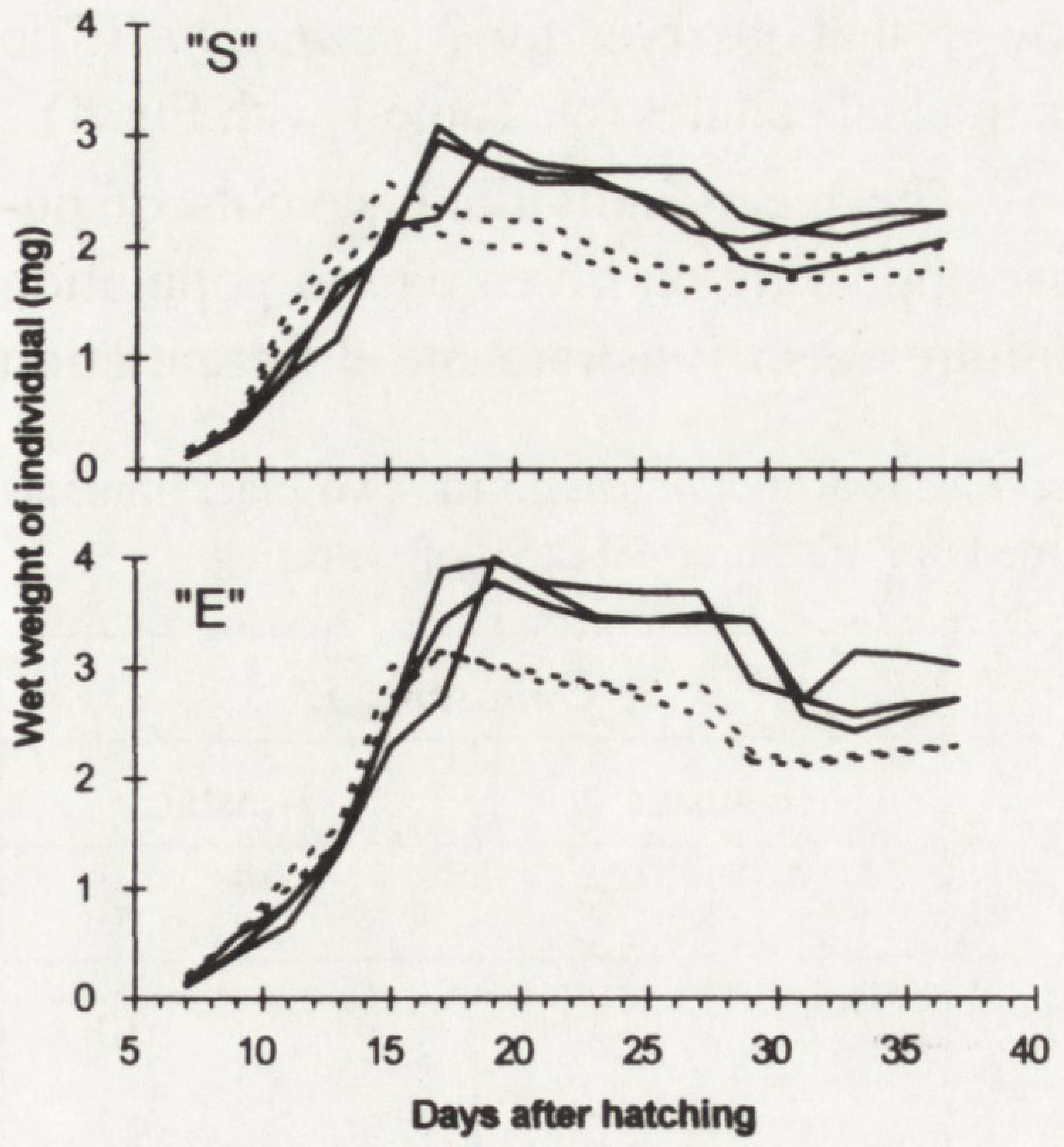

Fig. 1. Growth curves for male individuals of *T. castaneum* sooty ("S") and *T. confusum* ebony-2 ("E") selected to exemplified the variation between 7-instar (solid line) and 6-instar (dashed line) ecotypes

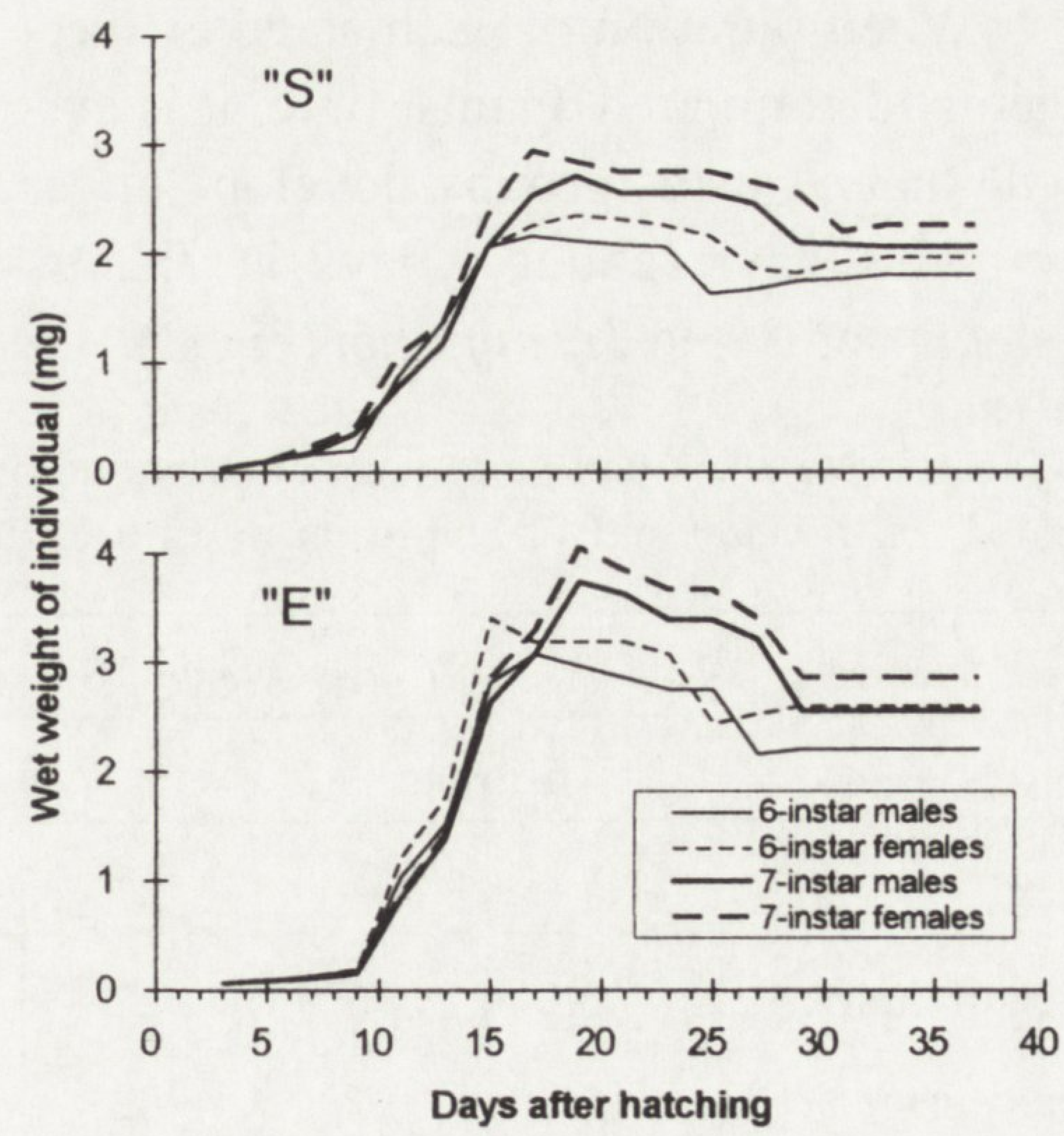

Fig. 2. Average growth curves of black mutants of *T. castaneum* sooty ("S) and *T. confusum* ebony-2 ("E").

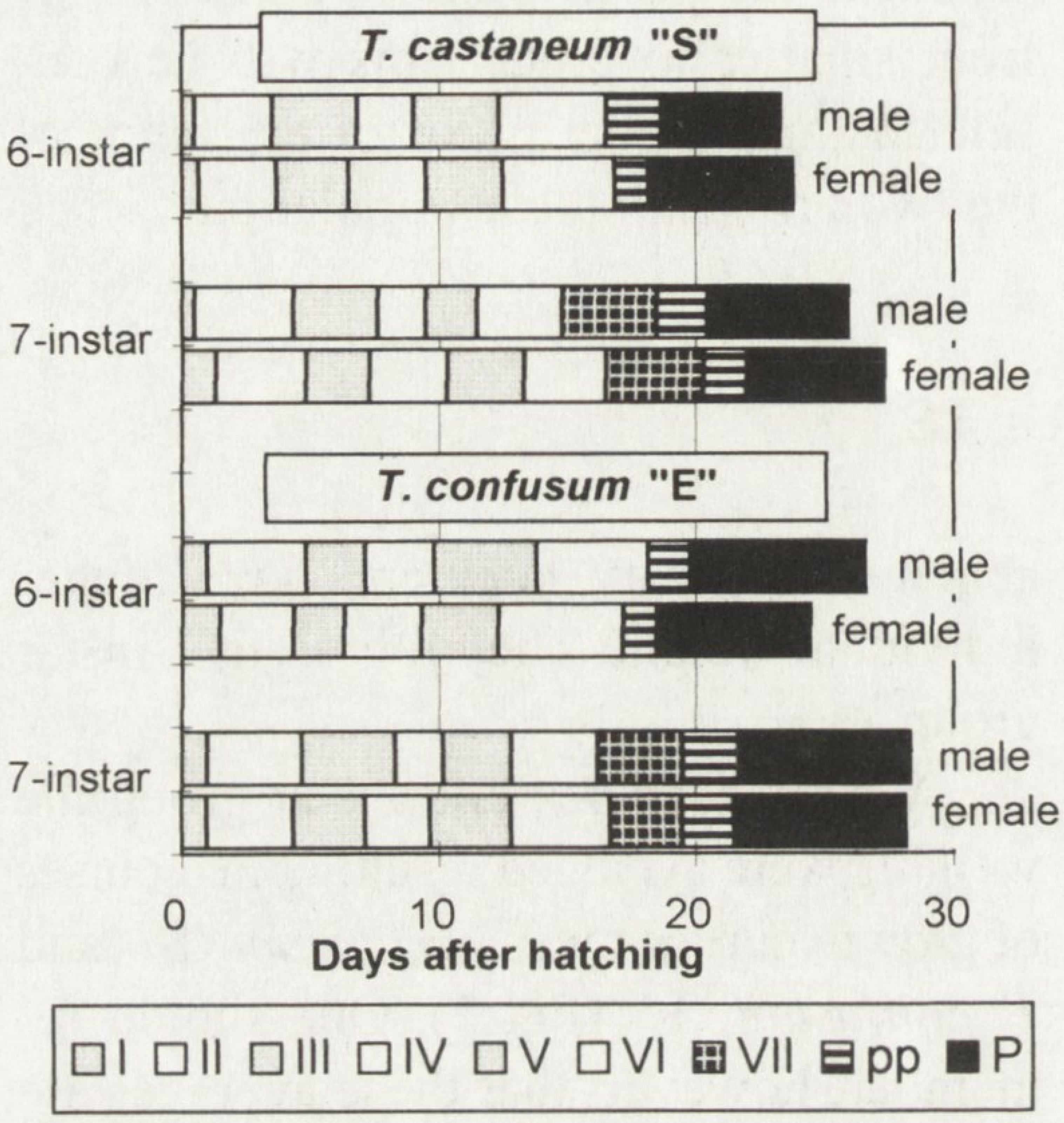

Fig. 3. Duration of developmental stages in two ecotypes of individuals of black mutants of *T. castaneum* sooty ("S") and *T. confusum* ebony-2 ("E").

star group, so the time of reaching maximum weight did not coincide (Fig. 2). The weight differences were of the order of 0.6mg in *T. castaneum* "S" and of 1.0 mg in *T. confusum* "E" (Fig. 2).

When duration of each stage is averaged and compared against time, it is evident that 7-instar groups develop longer by a few days (about 4 days in *T. castaneum* and 3 – in *T. confusum* (Fig. 3).

It is interesting to consider the share of these two groups in the populations of black mutants under study (Table 1). In *T. castaneum* "S" there were about 80% of individuals representing 7-instar group and 20% – 6-instar group. So the ratio of 7- to 6-instar group was 4:1. In *T. confusum* "E" the share of 6-instar individuals is much smaller, resulting in corresponding percentages of 94% and 6% giving the ratio of 7- to 6-instar as 15:1 (Table 1).

In the experiments aiming at determining frequency of pupal appearance in the population against time in both the strains it was found that the two black mutants show two-peak curves of frequencies of pupal appearance. In strain "S" of *T. castaneum* the duration of development to the moment of reaching pupal stage is the longest in the highest density (510 individuals per 32 g of medium) being shorter in both lower densities. (Fig. 4). The proportion between the two peaks appearing earlier and later seem to point to a somewhat lower share of 6-instars in both strains, but never as low as that observed for *T. confusum* "E" in individual cultures (cf. Table 1 with Fig. 4).

The mean individual weights of pupae appearing on given day in population cultures also illustrate the differentiation

Table 1. Numbers and percentage contribution of 6- and 7-instar individuals in the two black mutants of *T. castaneum* and *T. confusum* in 32 g cultured medium and after 30 days of culturing

Species	*T. castaneum* "S"				*T. confusum* "E"			
instar group	6-instar		7-instar		6-instar		7-instar	
sex	♂	♀	♂	♀	♂	♀	♂	♀
no. of indiv.*	5	6	22	21	2	1	17	30
percentage	18	22	82	78	10	3	90	97
% when sex ignored**	20		80		6		94	

* – Absolute numbers represent distribution of initial 60 single cultures in which individuals reached pupal stage.

** – In each strain each sex is considered as hundred per cent if sex is not ignored

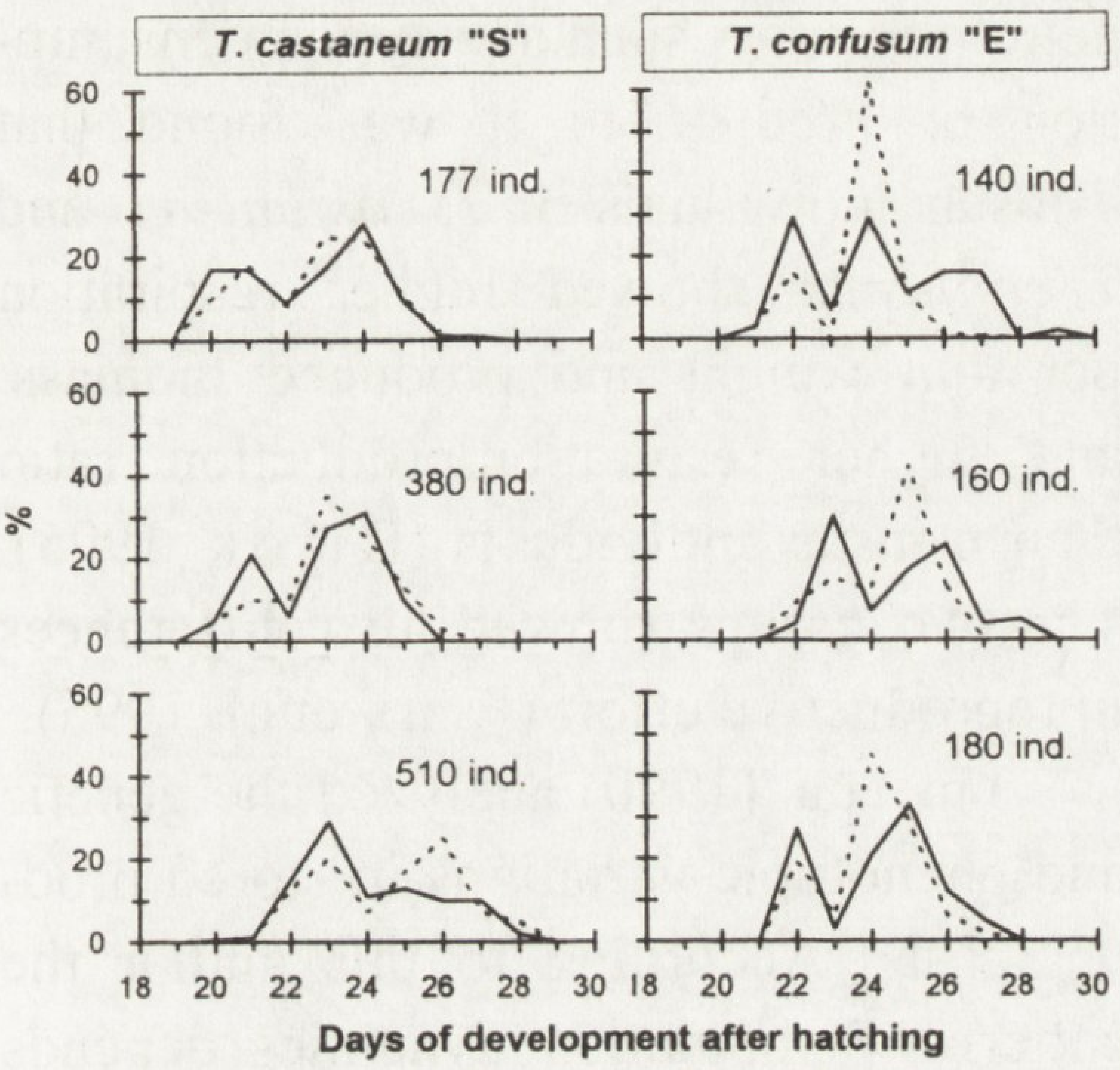

Fig. 4. Percentage of pupae appearing on a given day of development out of total number of individuals of each sex (= 100%) reared each in 32 g of medium in synchronized cultures of black mutants of *T. castaneum* sooty ("S") and *T. confusum* ebony-2 ("E") (dotted lines – females; solid lines – males)

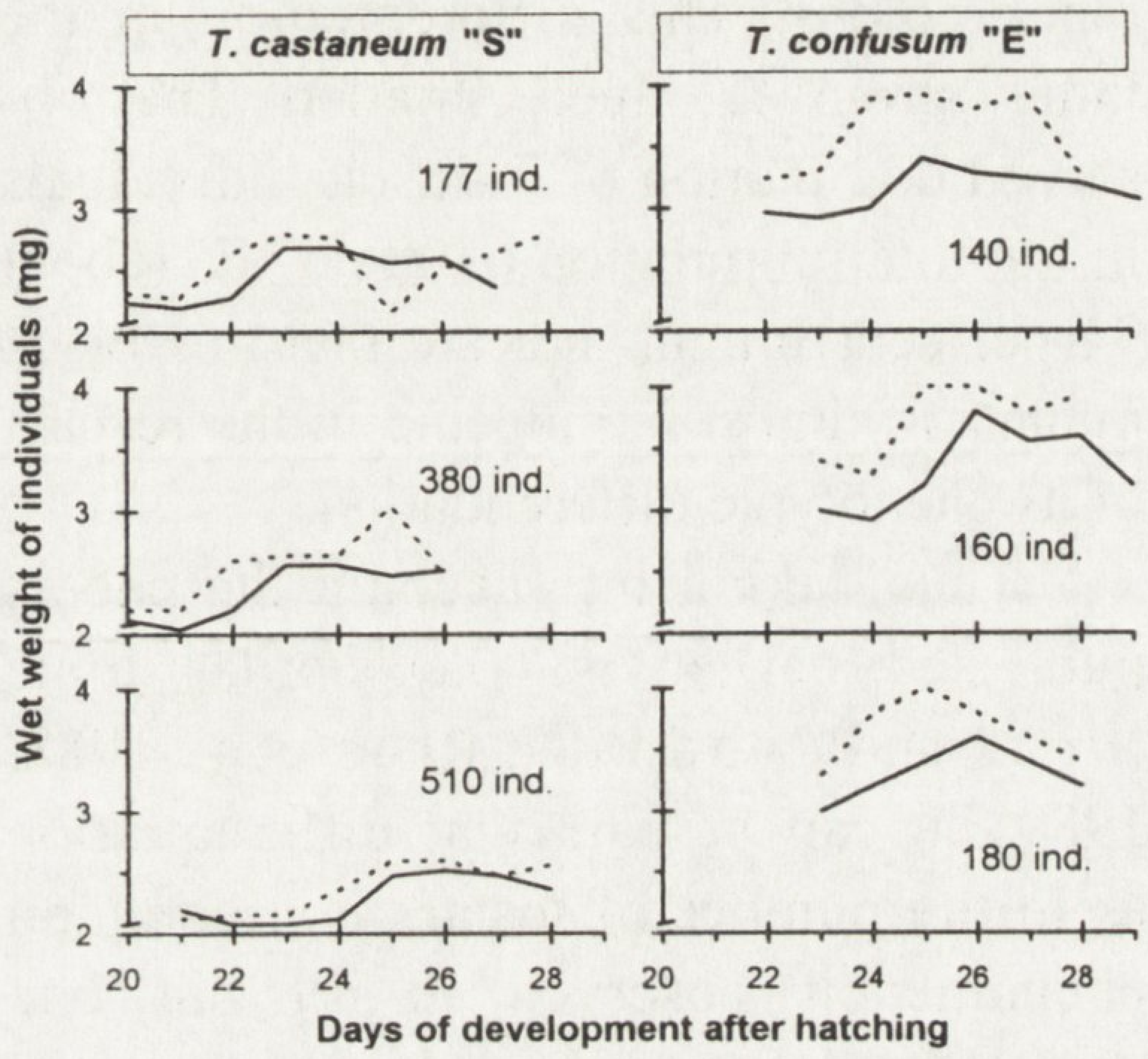

Fig. 5. Average weight of pupae appearing on a given day of development in synchronized cultures of black mutants of *T. castaneum* sooty ("S") and *T. confusum* ebony-2 ("E") reared at different numbers at 32 g of medium (dotted lines – females; solid lines – males)

of these strains. The earlier appearing pupae were, as a rule, lighter than those appearing later (Fig. 5). Female pupae were usually heavier than male pupae which was especially true for *T. confusum* "E" strain.

4. DISCUSSION

According to Sokoloff et al. (1960) black mutant "b" of *T. castaneum* is semidominant mutation showing no differential viability between the different genotypes. Reciprocal crosses showed that this gene is most linked with sex, but is autosomal. The strain tested in the present paper as "S" is recessive (see Sokoloff 1966 for further references). The other strain tested here, "ebony-2" is a *T. confusum* recessive mutant found by Dyte and Blackman in 1961 (Sokoloff 1966). Its phenotype is said to be identical with the semidominant "b", but tests of allelism showed that they were not allelic (e-2 is on linkage group II, near pearl; "b" is on linkage group III (Sokoloff – personal communication).

The observed proportions between 6- and 7-instar group in the two black mutants tested point to much higher homogeneity of *T. confusum* strain "E" (only 3 individuals turned to be 6-instars out of 60 tested) than of *T. castaneum* "S" strain (about 20% of tested individuals were of 6-instars).

The conclusion that can be drawn from the observed facts is as follows: "sooty" strain of *T. castaneum*, being a recessive strain for blackness, shows greater differentiation than strain "E" of *T. confusum*, which is also recessive for black colour. This differentiation in the latter strain is much smaller. The observed domination of 7 -instar individuals in the total population of strain "E" (about 94 per cent) might indicate that this black mutant is almost homogenic in the respect of its phenotypic differentiation.

Thus, taking into account the papers published earlier on the similar topic (Prus and Prus 1987, Prus et al. 1988, 1989), it can be said that differentiation as to the number of instars is a common phenomenon observed in all but one strains that has been tested so far, namely in: strain cI of *T. castaneum* (Prus 1976), cIV (Prus and Prus 1990, Prus 1993) "sooty" (present paper); in strain bIV of *T. confusum* (Bijok 1986) and "E" (present paper). Only strain bI showed no such differentiation (Prus and Prus 1990). Such commonly observed intrapopulation differentiation – perhaps occurring in majority of *Tribolium* strains which should be considered as most homogenous ones. Constant occurrence of 6- and 7-instar individuals in a *Tribolium* population permits for different responses of these groups to various environmental factors such as, for example, response to an inhibitory salt, e.g. tricalcium phosphate (Prus 1989, Prus 1995), to altered medium, e.g. wheat flour alone *versus* enriched with yeast (Prus in print), to conditioning and density (Bijok in print). The intrapopulation differentiation in *Tribolium* strains was further investiged in terms of energetic indicators such as oxygen consumption or production. It was found that 7-instar individuals of *T. castaneum* and *T. confusum* showed higher respiration per unit weight and produced biomass, and higher respiration/production ratio than 6-instar individuals (Bijok 1995). The two groups showed also differences in reproductive effort (Prus et al. 1995).

Imura (1990) analyzed the genetic and phenotypic variations in stored product pests. According to this author the pattern of population dynamics depends on types of life history of the species. Due to the fact that stored products are a nonrenewable resource, the insect populations are inevitably subjected to decline in number after establishment. When the population declines selection would favor long-lived and iteroparous individuals (Mertz 1971).

Similar approach to the role of environmental impact on phenotypic differentiation is that represented by McKee and Ebert (1996) working with *Daphnia magna* and Stam et al. (1996) working with parthenogenetic collembolans *Folsomia candida* (Willem).

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5. SUMMARY

The two groups of 6- and 7- instar individuals were found in each strain of black mutant "S" *T. castaneum* and "E" *T. confusum*. The differences were concluded on the basis of growth curves studed in detail (Fig 1). In strain "E" of *T. confusum* the share of 6-instar individuals was much smaller than that of strain "S" of *T. castaneum* (Table 1).

In strain "S", the two discerned groups differred foremostly by weight, 6-instar individuals being lighter than 7-instar ones, but

their growth curves rather coincided with time, whereas in "E", the maximum body weights were observed earlier in the development of 6-instar than of 7-instar group (Fig 2).

From the average duration of each stage, as well as total development time it became evident that individuals of 7-instar group developed longer by about 4 days in "S" strain and about 3 days in "E" strain than in respective 6-instar groups (Fig. 3).

The experiments concerning frequency of pupal appearance in synchronized cultures of both the strains proved that two peaks of occurrence of pupae are typical for both strains. This corroborates phenotypic differentiation of these strains (Fig. 4). Lower individual weights of pupae appearing earlier and heavier pupae appearing later also confirmed this conclusion (Fig. 5).

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