BIOLOGICAL COMPARISON OF DIFFERENT POPULATIONS OF ARGAS (PERSICARGAS) PERSICUS (OKEN)

F. DUSBAÈEK
Institute of Parasitology, Czechoslovak Academy of Sciences, České Budìjovice

Abstract. Characteristic features of the developmental cycle of Argas (P.) persicus (Oken, 1818) populations and their hybrids from Czechoslovakia and Azerbaijan were compared. It was proved that the phenotypic differences found earlier (Dusbaëek 1984) are also accompanied by genotypic differences reflected in the peculiarities of developmental cycle. However, no traces of genetic incompatibility were detected. The heaviest losses in the course of developmental cycle were found in homogamic crosses and are considered to be due to the inbreeding depression during laboratory culturing. Some biological properties seem to have been inherited in the matroclinial form and may therefore be connected with extrachromosomal inheritance.

Experimental interspecific hybridization is one of the basic methods of biological analysis of the systematic validity of related species. The study of a species at the level of gamma-taxonomy (Mayr 1969) i.e., at the intraspecific level, requires a deeper analysis of interpopulation relationships (McDonald 1976). Remarkable results were obtained when developmental cycles of different argasid populations were compared, different populations were crossbred and the geographic variability of the phenotype was studied.

Balashov (1971), while crossbreeding 14 different populations of Ornithodoros (Pavlovskyella) tartakovskyi Olenev, 1931, in some combinations found a modified fertility in F1 generation of hybrids, manifesting itself in a lesser number of eggs, unfinished embryogenesis, reduced viability of larvae and even complete genetic incompatibility in several crosses. These biological differences were much more distinct than the differences in the phenotype of individual populations (Balashov 1972, 1975a). During similar crossbreedings of the F1 generation of hybrids in Ornithodoros (Pavlovskyella) serracoum Olenev, Zassukhin et Fenyuk, 1934 a slight genetic incompatibility appeared between the northern and southern groups of populations, but it was lacking inside these groups (Balashov 1975b). Hoogstraal et al. (1975) found differences in the course of developmental cycle in five studied populations of Argas (Persicargas) robertsi Hoogstraal, Kaiser et Kohls 1968, and likewise did Gothe et Koop (1974), who studied pre-larval stage in reciprocal hybrids of two populations of Argas (Persicargas) arboreus Kaiser, Hoogstraal et Kohls, 1964.

The studies on geographic variability of phenotype in three populations of Argas (Persicargas) persicus (Oken, 1818) revealed that out of 48 randomly selected characters these populations were similar only in 21 of them (Dusbaëek 1984). Therefore, we decided to compare biologically two of these three populations, in order to find genetic differentiation of species and to establish the relationship between the phenotype and genotype in these populations.
MATERIALS AND METHODS

The A. peregrina populations from Czechoslovakia and Azerbaijan were used for biological comparison. The former one was collected from henshouses in the village Peřeň in Sokolovo district of Levine, Czechoslovakia, on 28 June 1972. The second one was obtained by courtesy of RNDr. J. Želevsky, D.Sc., of the Virologisches Institute of the Slovak Academy of Sciences in Bratislava, and was collected from henshouses in the villages Ardel and Shiklyan of the district Yevlakh, the Azerbaijan SSR, on 16 and 22 October 1972. The populations of both species were colonized for a number of years and several generations in the laboratory at temperature of 27 ± 1°C and 72 ± 3% RH in darkness. Our experiment was conducted under the mentioned conditions.

The biological comparison of populations consisting of homogeneous and heterogenous crosses of adult flies was conducted and parallel and was started on 15 June 1981. At the first stage, the individual pair was separated into two sets: the first one was in a wire cage above a dish filled with water. The males were used for oviposition only once. Nymphs and imagines were fed one month after the moult of last instar on older chickens and cockerels previously used for feeding, by method of Kaiser (1965). Evaluation was based on those specimens whose development continued following the first blood-meal. The duration of pre-oviposition was assessed from the day on which the engorged females and males were placed in pairs in culture jars, after the first eggs appeared, and the duration of pre-oviposition from the first eggs laid until the appearance of first larvae. As diapausing specimens were considered those argasses whose pre-oviposition period in the winter months exceeded 30 days. As the onset of diapause was considered the date of last feeding, as the end of winter diapause was regarded the molting of the next developmental stage.

The total recorded number of F1 generation consisted of 2,822 eggs, 1,500 unfed larvae, 764 engorged larvae, 626 nymphs I, 483 nymphs II, 80 nymphs III, 3 nymphs IV, 294 and 219. The described material of F1 generation included 5,859 eggs and 4,252 unfed larvae.

For statistical evaluation of differences in the duration of individual stages of development and further quantitative characters in homogeneous and heterogenous crosses of parents (Tables 1 and 2) Student's t-test was used, while the comparison of diapauing nymphs I and II and A. peregrina were carried out by one sample t-test. The differences in viability of the prelact and larval developmental stages of F1 generation (Tables 4 and 5) were compared by the statistical test equality of parameters of Poisson random variables after Sushkamo and Hafl (Fabini, 1983) and Student's t-test, for the comparison of the duration of pre-oviposition and pre-egg stage Student's t-test was used. For the implementation of these tests the author is indebted to RNDr. J. Želevsky, D.Sc., the Institute of Biometrics of the South Bohemian Biological Centre at Česká Budějovice. The following abbreviations are used in the text and tables:

Aa = progeny of A from Azerbaijan and a from Czechoslovakia
Ac = progeny of A from Azerbaijan and c from Czechoslovakia
Cc = progeny of c from Azerbaijan and c from Czechoslovakia
Ca = progeny of c from Azerbaijan and A from Czechoslovakia

RESULTS

The results obtained by biological comparison of populations are summed up in Tables 1—5. The duration of respective developmental stages (Table 1), the egg output of developing larvae and nymphs (Table 2), and number of diapauing specimens (Table 3) were studied in parental generations. Only pre-oviposition and larval stages of development (Tables 4 and 5) were compared in filial generations.

Table 1. Duration of periods in the life cycle of homogeneous and heterogenous crosses of Azerbaijan and Czechoslovak populations of Argyus peregrina (in days, ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Aa</th>
<th>Ac</th>
<th>Ce</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Pre-oviposition</td>
<td>17.0 ± 8.3</td>
<td>18.4 ± 8.4</td>
<td>16.0 ± 8.4</td>
<td>17.0 ± 10.0</td>
</tr>
<tr>
<td>Egg Incubation</td>
<td>14.0 ± 2.9</td>
<td>16.0 ± 2.4</td>
<td>18.0 ± 2.9</td>
<td>16.0 ± 2.9</td>
</tr>
<tr>
<td>Larva Feeding</td>
<td>4.9 ± 0.6</td>
<td>4.5 ± 0.5</td>
<td>5.2 ± 0.5</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>Prolongement</td>
<td>10.6 ± 3.1</td>
<td>11.8 ± 3.8</td>
<td>9.0 ± 1.8</td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td>N I Prolongement</td>
<td>17.8 ± 14.2</td>
<td>20.7 ± 15.2</td>
<td>16.3 ± 14.6</td>
<td>16.4 ± 13.0</td>
</tr>
<tr>
<td>N II Prolongement to N III</td>
<td>19.3 ± 9.5</td>
<td>14.1 ± 0.5</td>
<td>14.3 ± 2.0</td>
<td>17.2 ± 4.5</td>
</tr>
<tr>
<td>N III Prolongement to N IV</td>
<td>20.7 ± 7.3</td>
<td>18.1 ± 2.6</td>
<td>17.5 ± 2.1</td>
<td>17.1 ± 1.4</td>
</tr>
<tr>
<td>N IV Prolongement to N V</td>
<td>22.0 ± 0.0</td>
<td>16.0 ± 0.0</td>
<td>19.0 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Productivity of different developmental stages in the life cycle of homogeneous and heterogenous crosses of Azerbaijan and Czechoslovak populations of Argyus peregrina

<table>
<thead>
<tr>
<th></th>
<th>Aa</th>
<th>Ac</th>
<th>Ce</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg Mean number in a batch</td>
<td>118.5 ± 51.1</td>
<td>180.0 ± 61.1</td>
<td>124.0 ± 33.3</td>
<td>147.2 ± 12.6</td>
</tr>
<tr>
<td>Larva with batched</td>
<td>59.0 ± 8.9</td>
<td>80.0 ± 9.7</td>
<td>39.0 ± 3.7</td>
<td>46.0 ± 2.1</td>
</tr>
<tr>
<td>% of fed</td>
<td>53.9</td>
<td>46.9</td>
<td>37.3</td>
<td>40.0</td>
</tr>
<tr>
<td>% of moulted</td>
<td>91.5</td>
<td>76.0</td>
<td>49.4</td>
<td>80.8</td>
</tr>
<tr>
<td>N I % of moulted</td>
<td>96.7</td>
<td>68.8</td>
<td>50.0</td>
<td>87.3</td>
</tr>
<tr>
<td>N II % of moulted to N III</td>
<td>18.2</td>
<td>16.8</td>
<td>40.4</td>
<td>21.9</td>
</tr>
<tr>
<td>N III % of moulted to N IV</td>
<td>40.9</td>
<td>44.5</td>
<td>25.5</td>
<td>30.5</td>
</tr>
<tr>
<td>% of total productivity</td>
<td>32.2</td>
<td>31.9</td>
<td>10.4</td>
<td>32.0</td>
</tr>
</tbody>
</table>

a) Homogenic and heterogenous crosses of parents (P)

A comparison of the duration of respective developmental stages in homogenic and heterogenous crosses (Table 1) shows a conspicuous agreement in most indices and only minimum statistically significant differences. The most conspicuous differences appeared in the preoviposition period of nymphs I, which significantly differed (P < 0.01) in most of the combinations tested. These differences are consistent with the unequal number of engorged nymphs I in respective crosses which have entered the winter diapause and with the relevant prolongation of the diapause period. While the differences in the number of diapauing and non-diapauing nymphs I between crosses Cc and Ca proved to be not significant, the differences in the remaining crosses were statistically significant (P < 0.01) (Table 3). The diapause of nymphs I in the cross Cc occurred between 22 October 1981 and 20 April 1982, in the cross Ac between 16 October 1981 and 15 April 1982, in the cross Cc between 9 December 1981 and 8 March 1982 in the cross Aa between 16 November 1981 and 22 February 1982. In crosses Aa and Ac the period of
The differences in the egg output (Table 2) between respective crosses were not significant, but the two homogamic crosses (Aa and Cc) significantly differed (P < 0.01) in a lesser number of hatched larvae. In comparison with Table 5 where crosses Aa and Cc served as controls, it is evident that the lesser number of hatched larvae also occurred in these crosses. The cross Cc yielded explicitly a lower percentage of engorged larvae (P < 0.05) than the cross As and a significantly lower (P < 0.05) production of nymphs I and II (Table 2). There were no significant differences in the percentage of moulting nymphs II and III, only in the number of nymphs II moulting to nymphs III there was a significant difference between crosses Cc and Ac. All these differences resulted in a lower total productivity of homogamic cross Cc, representing the percentage of unfed larvae able to complete their development to imago.

b) Brother-sister crosses and backcrosses of F1, hybrids

The investigation of crosses in F1 hybrid generation was restricted to the studies on prelarval and larval stages of development only (Tables 4, 5). The period of the preoviposition was significantly shorter (P < 0.01) in all three crosses of females from the Czechoslovak tick population (Cc × Cc, Cc × Ca, Cc × Ac), similarly as in crosses Ca × Cc. In other crosses there were no significant differences in the duration of preoviposition. Likewise in the duration of pre-eclosion the individual crosses showed no significant differences.

Table 4. Duration of periods in prelarval stage of life cycle and survival of larvae of brother-sister crosses and backcrosses of F1, hybrids of Azerbaijan and Czechoslovak populations of *Argos perpus* (in days, x ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Pre-oviposition</th>
<th>Pre-eclosion</th>
<th>Survival of larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>As × As čř</td>
<td>17.0 ± 10.6</td>
<td>16.5 ± 2.4</td>
<td>88.5 ± 38.2</td>
</tr>
<tr>
<td>As × Ac čř</td>
<td>16.7 ± 3.8</td>
<td>15.0 ± 0.8</td>
<td>124.5 ± 36.9</td>
</tr>
<tr>
<td>As × Čc čř</td>
<td>26.2 ± 13.9</td>
<td>15.6 ± 2.5</td>
<td>94.2 ± 43.1</td>
</tr>
<tr>
<td>As × Ča čř</td>
<td>19.2 ± 6.7</td>
<td>17.9 ± 1.6</td>
<td>134.6 ± 52.7</td>
</tr>
<tr>
<td>Ac × Čc čř</td>
<td>26.8 ± 16.3</td>
<td>15.2 ± 1.8</td>
<td>102.3 ± 28.6</td>
</tr>
<tr>
<td>Ac × Ča čř</td>
<td>19.8 ± 3.8</td>
<td>15.2 ± 1.1</td>
<td>139.0 ± 31.7</td>
</tr>
<tr>
<td>Čc × Čc čř</td>
<td>8.5 ± 1.7</td>
<td>20.3 ± 2.1</td>
<td>210.5 ± 10.4</td>
</tr>
<tr>
<td>Čc × Ča čř</td>
<td>11.2 ± 2.9</td>
<td>17.4 ± 1.9</td>
<td>41.4 ± 15.4</td>
</tr>
<tr>
<td>Ča × Čc čř</td>
<td>11.5 ± 0.9</td>
<td>17.3 ± 1.3</td>
<td>40.0 ± 13.5</td>
</tr>
<tr>
<td>Ča × Ča čř</td>
<td>13.2 ± 3.3</td>
<td>16.2 ± 1.8</td>
<td>130.4 ± 51.6</td>
</tr>
<tr>
<td>Ča × As čř</td>
<td>22.6 ± 10.6</td>
<td>17.2 ± 2.3</td>
<td>224.4 ± 39.6</td>
</tr>
<tr>
<td>Ča × Ča čř</td>
<td>19.2 ± 3.2</td>
<td>16.0 ± 2.0</td>
<td>133.2 ± 31.8</td>
</tr>
</tbody>
</table>

diapause of nymphs I was consequently shorter and its onset considerably delayed. The preomouling period of nymphs II, moulting to nymphs III, females and males was definitely longer in the cross As (P < 0.01), similarly as in nymphs III moulting to females and males but only at the level of P < 0.05. Also here it became evident that only in this cross the winter diapause of nymphs II occurred (Table 3). The pre-oviposition in crosses Cc and Ca was shorter than in crosses As and Ac, but only the shorter duration of pre-oviposition in the cross Ca was significant (P < 0.01). In this cross and in the cross Cc as well, also the premouling period in larva (P < 0.01) was significantly shorter than in crosses Ac and As, while crosses Cc and Ca did not differ from each other significantly, similarly as the differences between As and Ac were not significant.

Table 5. Fertility of brother-sister crosses and backcrosses of F1, hybrids of Azerbaijan and Czechoslovak populations of *Argos perpus* (*Mean egg number in a batch, % of hatched larvae, % of unhatched developed larvae, % of sterile eggs*)

<table>
<thead>
<tr>
<th></th>
<th>Mean egg number in a batch</th>
<th>% of hatched larvae</th>
<th>% of unhatched developed larvae</th>
<th>% of sterile eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>As × As čř</td>
<td>91.0 ± 52.3</td>
<td>54.4</td>
<td>12.1</td>
<td>33.5</td>
</tr>
<tr>
<td>As × Ac čř</td>
<td>113.3 ± 58.5</td>
<td>92.9</td>
<td>4.0</td>
<td>3.1</td>
</tr>
<tr>
<td>As × Čc čř</td>
<td>87.8 ± 51.7</td>
<td>94.5</td>
<td>1.1</td>
<td>4.4</td>
</tr>
<tr>
<td>As × Ča čř</td>
<td>87.0 ± 42.0</td>
<td>89.4</td>
<td>4.1</td>
<td>6.5</td>
</tr>
<tr>
<td>Ac × Čc čř</td>
<td>91.0 ± 33.1</td>
<td>87.0</td>
<td>1.5</td>
<td>10.6</td>
</tr>
<tr>
<td>Ac × Ča čř</td>
<td>88.8 ± 32.7</td>
<td>89.9</td>
<td>5.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Čc × Čc čř</td>
<td>96.0 ± 34.7</td>
<td>49.0</td>
<td>32.7</td>
<td>18.3</td>
</tr>
<tr>
<td>Čc × Ča čř</td>
<td>122.0 ± 33.3</td>
<td>67.2</td>
<td>29.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Ča × Čc čř</td>
<td>140.0 ± 31.6</td>
<td>67.0</td>
<td>67.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Ča × Ča čř</td>
<td>107.8 ± 63.7</td>
<td>95.2</td>
<td>6.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Ča × As čř</td>
<td>138.2 ± 51.4</td>
<td>91.5</td>
<td>4.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Ča × Ča čř</td>
<td>104.8 ± 48.5</td>
<td>91.0</td>
<td>4.2</td>
<td>4.8</td>
</tr>
</tbody>
</table>

The comparison of the egg output in F1, experimental crosses mostly revealed a lesser number of eggs in batches laid by females from Azerbaijan and by females coming from crosses of females Aa with different males. On the other hand, in females Cc and Ca the numbers of eggs were significantly higher (P < 0.01) in most cases. The only exception was the backcross As × Ac, where the number of eggs was significantly higher (P < 0.01) than in the other females of the group Aa and Ac, between which no significant differences appeared. In the control crosses Cc × Cc the number of eggs was significantly lower (P < 0.01) than in the other crosses in female groups Cc and Ca, which showed no significant mutual differences in the number of eggs.

258

259
The percentage of hatched larvae in most crosses was approximately at a same level (97.9—95.2 %). Only the two control crosses (As x As and Cc x Cc), showing no significant differences between each other, and across controls with females from Czechoslovak populations (Cc x Ca and Cc x Ac) differed significantly from remaining crosses in the lesser number of hatched larvae. The cross Cc x Ca yielded a significantly higher number of hatched larvae than crosses Cc x Cc and Cc x Ac which showed no significant mutual differences.

Differentiated were also the eggs in which the development had started but no hatching of larvae occurred from sterile eggs where the embryo did not develop at all. While the number of unhatched developed larvae was significantly higher in those crosses, in which the number of hatched larvae was significantly lower (As x As, Cc x Cc, Cc x Ca, Cc x Ac, and Cc x Cc), the number of sterile eggs was significantly higher only in the two control crosses, namely As x As and Cc x Cc.

The comparison of the life span of larvae clearly demonstrated the lower viability of the progeny of females from the Czechoslovak population (Cc x Cc, Cc x Ca, Cc x Ac) which survived a shorter period (P < 0.01) than the progeny from other crosses. Likewise the larvae from crosses As x As and As x Ca survived longer than those from As and Ac, but the difference was not significant; however, the survival of larvae from females Cc, the larvae from females As survived significantly longer (P < 0.01).

**DISCUSSION**

Although the development cycle of *Euglena persica* has been in recent years revised in populations from different geographic regions (Frolow 1970a, b, Frolow et Dzheev 1970a, b, Frolow et Kachekova 1975, Gothe and Koop 1974, Petrov and Gecheva 1975, Dushibek and Rosicky 1976, Khalil 1978, El Kamah and Wahab 1979, Srivastava et al. 1981), the results were obtained by Huret and Drumm (1988) and by the laboratory and field populations of *Euglena persica* *L. (1758)* and noted that most reproduction characteristics of wild females with colony males were similar to those of wild females with wild males; and colony females with wild males had reproduction characteristics similar to those of colony females with colony males. Consequently any environment passed on to progeny in the form of matroclinny and thereby connected with cytoplasmic heredity, may be involved here.

The characteristic features of viability of the individual developmental stages and the total productivity (summed up in Table 2) indicate the heaviest losses suffered during ontogenesis in cross Cc, in the percentage of hatched larvae as well as in cross As, consequently in both homogamic crosses. In the first series of experiments this phenomenon did not become manifest in heterogamic crosses (Table 3), but it did manifest itself again in homogamic backcrosses of F2 generation of pure males with pure females (Tables 4, 5) in a lesser average number of eggs laid in the cross Cc x Cc, as opposed to heterogamic crosses of females with different males, and also in lower percentages of hatched larvae in homogamic crosses As x As and Cc x Cc, as well as in a shorter life span of any early stages of the female group Cc. These characteristics may be connected with long-term inbreeding of a small number of males in the laboratory colony, which is reflected in the manifestation of recessive subtelth and lethal genes, resulting in a lower viability of the laboratory population (McDonald 1970b). Similar conclusions were drawn by Steward et al. (1986) after interpreting their results in terms of cycles of female and laboratory populations of *Boophilus microplus* (Canestrella, 1888).

It may be therefore concluded that the phenotypic differences found in the argad populations from Czechoslovakia and Azerbaijan (Dushibek 1984) are also accompanied by differences in genotype. However, no traces of genetic incompatibility were detected. The heaviest losses in the course of preimaginal cycle were found in homogamic crosses and as a result of inbreeding during laboratory culturing. Some biological characteristic features seem to have been inherited in the matroclinny form and may be connected with extrachromosomal inheritance.
BIOLÓGÍČOS SÍVARÍNEH RÁZNÝPI PÓPÚLACEI ARGAS (PERSCARCÁS) PERSCUÍS (OKEN)

RÁSME. Dívo súčasné kariéry a spôsoby rozvitalia Argas (P.) perscicus (Oken, 1818) z Cheshlovínie a z Česko-Slovenska. Uvádza, že džsebo, obnovovaného rozvitalia osekov (Dyubak, 1984) prekryva dvanásť zo súčasne-
miami genetickým súkromom v osobnostiach súčasnej rovnice. Osa trouby, ke by obnovované-
nie prekryva genetickou genetickou nekompatibilitat. Samce malej potreby v témpe súčasnej rovnice obnovovaného genetického skrýchovania a výskytu normálního forem ať by súčasnej genetickou nekompatibilitat.

REFERENCES

—Geographic variability of Ornithodoros tarto-


EL KAMAIH K. M., WAHAB K. S. A., Ar-


GOTHE R., KOOP E., Zur biologischen Be-
wesen der Validitiät von Argas perscicus (Oken, 1818). Arbus orborus Kaizer, Hoog-

HOOGSTRAAL H., QUIROIGS S. S., KHALIL G. M., KAISER M. N., The subgenus Per-

HUNT L. M., DRUMMOND R. O., Effect of laboratory rearing on the reproductive bi-


SRIVASTAVA S. C., KHAN M. H., MION

STEWARD N. E., CALLOW L. L., DUN-