Inheritance of body and testis size in the bushcricket *Poecilimon veluchianus* Ramme (Orthoptera; Tettigoniidae) examined by means of subspecies hybrids

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The inheritance of three parameters of body size: body mass, hind femur length and pronotum length, and of three measurements of male fertility: testis mass, relative testis mass (as part of body mass) and sperm number per spermatophore, were examined in crosses between the bushcricket subspecies *Poecilimon veluchianus veluchianus* and *P. v. minor*. Body size parameters differed surprisingly in their apparent genetic control: the genes determining pronotum length were autosomal, hind femur length was probably partly X-chromosomal and body mass was largely determined by genes on the X-chromosome. I consider whether sexually selected traits may commonly be sex-linked. Testis mass in the hybrid males was clearly different in reciprocal hybrids and was similar to the father's subspecies in both cases. As a result of the X-chromosomal (maternal) inheritance of body mass and a testis mass similar to the paternal subspecies, the relative testis mass in the hybrid males was larger or smaller than in the pure subspecies. In the male hybrids with reduced testis mass, the mean number of sperm was strongly affected. About 90% of these males transferred only a few sperm per spermatophore.

ADDITIONAL KEY WORDS:--bushcrickets – heredity – sex-linkage – hybrid dysgenesis – sperm number.

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INTRODUCTION

In many insect species body size has important consequences for reproduction. Especially male mating success and number and size of the female's eggs, all

three probably correlated with lifetime reproductive success, are largely
influenced by size (Thornhill & Alcock, 1983).

In bushcrickets body size has been shown to be subject to sexual selection both
in populations with a normal and a reversed operational sex-ratio (Gwynne,
1981, 1982). Male success in sperm competition largely depends on
spermatophore attachment time and spermatophore weight, both strongly
correlated with body weight (Wedell & Arak, 1989; Wedell, 1991; Reinhold &
Heller, 1993). Body size is also an important characteristic correlated with
female mating frequency (Heller & Helversen, 1991), female fecundity and
offspring size (Reinhold & Heller, 1993).

Although much is known about the role of body size in reproductive success of
bushcrickets, data regarding the genetic basis of size are almost lacking.
Information concerning the genetic basis of body size is, however, vital to our
understanding of the evolution and maintenance of these traits.

In this study the inheritance of body size was examined by means of subspecies
hybridization. Given two subspecies differ in size and can produce viable
hybrids, the body size of the resulting progeny may then allow some insight into
the heredity of the characteristics under consideration. Additional information
may be obtained from differences between the sexes of the resultant hybrids and
the direction of the crossing. By means of hybridization the genetics of such
diverse characteristics as bird migration (Berthold & Querner, 1981),
grasshopper stridulation (Helversen & Helversen, 1975a,b) and fruitley
morphology (Val, 1977; Templeton, 1977; Coyne, 1983) have been examined.

For examining the influence of size on reproductive success different size
measurements, including pronotum length, hind femur length and body mass,
were considered as measurements of bushcricket body size in the studies referred
to above. For this reason the heredity of all three parameters was examined in
this study of Poecilimon v. veluchianus Ramme, 1933 × P. v. minor Heller &
Reinhold, 1993, hybrids. These two subspecies of the bushcricket Poecilimon
veluchianus meet the requirements for a study of heredity of size since they
hybridize easily in the laboratory, produce viable offspring and have
conspicuously different body sizes.

As far as the function of reproductory organs is concerned, hybrids often show
dysfunction, a pattern that is nearly absent from other traits. The fertility of the
two sexes is often not affected symmetrically and it is the heterogametic sex that
normally suffers more from hybridization (Haldane’s rule, Haldane, 1922). In
accordance with this rule, the males of orthopteran hybrids (males are
heterogametic, normally XO) often only produce reduced numbers of sperm and
have testes of reduced size (for example: Hewitt et al., 1987). In order
to examine whether these two parameters were also affected in
P. v. veluchianus × P. v. minor hybrid males, the hybrids were compared with pure
strains.

METHODS

In this study we examined the inheritance of body and testis size parameters in
hybrids of two subspecies of Poecilimon veluchianus (P. v. veluchianus and
P. v. minor). These insects are medium-sized herbivorous bushcrickets endemic to
central Greece (Willems & Heller, 1992; Heller & Reinhold, 1993). We
examined insects from one population of each subspecies, close to the border
between the subspecies' ranges. The bushcrickets of the nominate subspecies *P. v. veluchianus* were from a population near the village of Vitoli and those of *P. v. minor* came from a population near the village of Tsouka (both in Nomos Fthiotis, Greece). The distance between the two populations is about 6.5 kilometers with no obvious geographical barrier.

Caged at their natural habitat in Vitoli the virgin females were randomly paired with males in the four possible combinations (*P. v. veluchianus* females with *P. v. veluchianus* and *P. v. minor* males; and *P. v. minor* females with *P. v. veluchianus* and *P. v. minor* males; between 16 and 48 females for each combination) and the eggs laid there were collected. For hibernation the eggs, separate for each female, were put on moist sand in petri dishes and held at 4°C. The following year the hatched larvae were bred in the laboratory at 23–28°C with a 12/12 h dark/light illumination cycle and fed with cabbage, *Taraxacum* and *Rabbus* leaves ad libitum. The larvae of the different families were housed separately, thus enabling a comparison of variance within and between families. The effect of a common environment should have had only a marginal influence on the between family variance, since the offspring of each female were distributed into several cages and the cages were repositioned almost daily during feeding.

Shortly after the adult moult, which occurred about 30 days after eclosion, each insect was marked individually with an adhesive label. In order to estimate sperm number, males that were older than 8 days and had not mated for at least 2 days were mated with virgin females. Shortly after copulation the spermatoaphore was removed with forceps and the spermatophylax, the gelatinous sperm-containing portion of the spermatoaphore, was separated from the sperm-containing ampulla. Subsequently the contents of the ampulla were suspended in 4 ml water by repeatedly passing it through a fine syringe. A haematocytometer (Neubauer, improved) was used for the estimation of sperm number per ampulla and the sperm were counted in a volume of 50 nl, or up to 16 times that volume if fewer than 100 spermatozoa were found.

The mass of each individual was measured to the nearest mg with a Mettler AE 50 balance. Only values from females that were adult for more than 8 days and from males which, in addition to this condition had not mated for at least 2 days, were included in the analysis. Some males of each combination were dissected and the mass of one testis was ascertained to the nearest 0.1 mg. Every individual was measured with dial calipers to the nearest 0.1 mm for dorsal pronotum and hind femur length. Insects collected in the field were examined for comparison with those bred in the laboratory for all measurements, except testis mass.

The analysis of within and between family body size variances revealed significantly higher variability between families (Table 1). Because the within family values were not independent, we used family means (separate for both sexes) in pairwise comparisons (Mann-Whitney U-Test).

**RESULTS**

**Body mass**

The mean body mass of males and females clearly differed between the subspecies (Fig. 1A) but the mean body mass of field collected (v, m, Fig. 1A)
Figure 1.
Figure 1. Mean body-size (± SE) in males (○) and females (□) of *P. v. veluchianus* (v: insects from the field, vv: laboratory bred insects), *P. v. minor* (m: insects from the field, mm: laboratory bred insects) and their hybrids (v v, m v, m m: offspring of *P. v. veluchianus♂ × P. v. minor♀, and m v, m m for the reverse cross) measured as body mass (A), pronotum length (B) and hind femur length (C). (n between 25 and 206 for the different values).

and laboratory bred insects (vv, mm, Fig. 1A) was very similar. The body mass of the female hybrids did not depend on the direction of the cross and was intermediate when compared to the pure subspecies with a certain dominance of *P. v. veluchianus* genes. The hybrid males of the two crosses, however, had a very different mean body mass (Table 2) that resembled the mean value of the males from the population of their mothers in both cases. The within family variance for body mass in *P. v. veluchianus* was not significantly smaller than the between family variance in both sexes (Table 1).

**Pronotum length**

In the larger subspecies *P. v. veluchianus*, the pronotum was significantly longer than in *P. v. minor* and in both subspecies the females had larger pronota than the males. The laboratory bred insects of the pure subspecies were smaller than those collected in the field (Fig. 1B), but the difference between *P. v. veluchianus* and *P. v. minor* was not influenced greatly because the reduction in size was similar in the two subspecies. The values for the pronotum length of the female and male hybrids were independent of the crossing-type and were more similar to the pronotum length of *P. v. veluchianus*, thus indicating that pronotum length is controlled by autosomal genes that are dominant in *P. v. veluchianus* (Table 2).
Table 1. Analysis of the variances of all three size measurements within and between families, for the two subspecies. (MS = mean square)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>d.f.</th>
<th>MS</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. veluchianus veluchianus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>♀ between families</td>
<td>24</td>
<td>16787</td>
<td>1.36</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>72</td>
<td>12327</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂ between families</td>
<td>28</td>
<td>9725</td>
<td>1.65</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>59</td>
<td>5912</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pronotum length</td>
<td>♀ between families</td>
<td>25</td>
<td>7.95</td>
<td>1.87</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>54</td>
<td>4.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂ between families</td>
<td>29</td>
<td>15.61</td>
<td>2.91</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>59</td>
<td>5.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind femur length</td>
<td>♀ between families</td>
<td>24</td>
<td>108.3</td>
<td>2.52</td>
<td>0.003</td>
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<tr>
<td></td>
<td>within families</td>
<td>49</td>
<td>43.0</td>
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<td></td>
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<tr>
<td></td>
<td>♂ between families</td>
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<td>66.4</td>
<td>2.09</td>
<td>0.010</td>
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<td></td>
<td>within families</td>
<td>54</td>
<td>31.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. veluchianus minor</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Body mass</td>
<td>♀ between families</td>
<td>11</td>
<td>13412</td>
<td>2.54</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>24</td>
<td>5282</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂ between families</td>
<td>14</td>
<td>3473</td>
<td>2.67</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>28</td>
<td>1301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pronotum length</td>
<td>♀ between families</td>
<td>12</td>
<td>8.44</td>
<td>1.53</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>20</td>
<td>5.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂ between families</td>
<td>15</td>
<td>7.58</td>
<td>2.06</td>
<td>0.056</td>
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<td>within families</td>
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<td>3.68</td>
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</tr>
<tr>
<td>Hind femur length</td>
<td>♀ between families</td>
<td>11</td>
<td>60.7</td>
<td>2.41</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>20</td>
<td>25.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂ between families</td>
<td>15</td>
<td>68.4</td>
<td>2.91</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>within families</td>
<td>22</td>
<td>23.5</td>
<td></td>
<td></td>
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</tbody>
</table>

In hybrid males the family means for pronotum length were not even distinguishable from that of the *P. v. veluchianus* males (Table 2).

**Hind femur length**

The length of the hind femora was larger in *P. v. veluchianus* than in *P. v. minor* in both sexes (Fig. 1C). The laboratory bred insects were smaller than the field-collected ones but the difference between the two subspecies remained consistent (Fig. 1C). The female hybrids of the two crosses had a similar hind femora length but the males differed slightly in respect to the direction of the crossing (Table 2). Males with a *P. v. veluchianus* mother had larger hind femora than males with a *P. v. minor* mother (Mann Whitney, *P* < 0.02).

**Sperm number**

Field collected *P. v. veluchianus* males produced spermatophores that contained about 10.5 million sperm (*SE* = 0.5 million) thus containing slightly more than spermatophores of *P. v. minor* males (7.5 million, *n* = 43, *SE* = 0.5, Mann-Whitney, *Z* = 3.75, *P* < 0.001). The males of the laboratory bred pure subspecies transferred spermatophores with a mean of 6.8 (*SE* = ±0.7, *n* = 36, *P. v. veluchianus*) and 7.6 (*SE* = ±1.0 *n* = 18, *P. v. minor*) million sperm respectively (Mann-Whitney, *Z* = 0.54, *P* > 0.5). Thus the spermatophores of
Table 2. Body size in the different crosses. For the comparison of the size parameters between the crossing types, family means were used. The distributions were pairwise compared using Mann-Whitney's U-test and the values marked with different letters are significantly different (the significance level $P = 0.05$ was adjusted to $P = 0.008$ in order to correct for the inflation of type-I error). The mean values shown here are average family means $\pm SE$ and the sample size in parentheses denotes the number of families for every crossing-type.

<table>
<thead>
<tr>
<th>Size-parameter</th>
<th>vel$\hat{o}$ × vel$\hat{o}$</th>
<th>vel$\hat{o}$ × min$\hat{o}$</th>
<th>min$\hat{o}$ × vel$\hat{o}$</th>
<th>min$\hat{o}$ × min$\hat{o}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>625 ± 12 (29) a</td>
<td>438 ± 19 (8) b</td>
<td>579 ± 15 (12) a</td>
<td>367 ± 11 (15) c</td>
</tr>
<tr>
<td>Pronotum length</td>
<td>5.1 ± 0.1 (30) a</td>
<td>4.9 ± 0.1 (8) a</td>
<td>5.0 ± 0.1 (12) a</td>
<td>4.3 ± 0.1 (16) b</td>
</tr>
<tr>
<td>Hind femur length</td>
<td>16.2 ± 0.1 (29) a</td>
<td>14.9 ± 0.3 (8) b</td>
<td>15.6 ± 0.1 (12) b</td>
<td>13.9 ± 0.2 (16) c</td>
</tr>
<tr>
<td>Female offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>736 ± 17 (25) a</td>
<td>655 ± 26 (8) ab</td>
<td>638 ± 21 (11) b</td>
<td>509 ± 20 (12) c</td>
</tr>
<tr>
<td>Pronotum length</td>
<td>5.4 ± 0.0 (26) a</td>
<td>5.2 ± 0.1 (8) b</td>
<td>5.1 ± 0.1 (12) b</td>
<td>4.7 ± 0.1 (13) c</td>
</tr>
<tr>
<td>Hind femur length</td>
<td>17.4 ± 0.1 (25) a</td>
<td>16.5 ± 0.3 (8) b</td>
<td>16.8 ± 0.2 (11) b</td>
<td>15.2 ± 0.2 (12) c</td>
</tr>
</tbody>
</table>

Laboratory bred $P. v. veluchianus$ males contained a reduced number of sperm compared to the field collected insects (Mann-Whitney, $Z = 3.7$, $P < 0.001$). The hybrid males with a $P. v. minor$ mother produced spermatophores with numbers of sperm comparable to the males of the pure species (7.3 ± 1.4 million). The hybrid males with a $P. v. veluchianus$ mother, however, transferred spermatophores with a mean of only 2.1 million sperm ($SE = 0.4$, $n = 32$, $P < 0.01$, for every comparison with Mann-Whitney). Comparing the

Figure 2. Frequency histogram of sperm number per spermatophore for the four types of males ($n = 81, 32, 12, 60$ for $vv + v$, $m_v$, $v_d m$, and $min + m$). $\bigcirc$ $P. v. veluchianus$; $\blacksquare$ $min \times vel$; $\square$ $vel \times min$; $\bigcirc$ $R. v. minor$. 
distribution of sperm number per spermatophore in the four male-types it is
evident that the reduced mean sperm number in hybrid males with a
*P. v. veluchianus* mother is largely due to males (see Fig. 2) transferring
spermatophores with few (< 1 million) or no sperm (in the spermatophores of 7
from 32 males not a single spermatozoon was found). The within family variance
in sperm number of the hybrid males of this cross was not significantly different
from the variance between families (Kruskal-Wallis ANOVA, values from 23
males of 10 families, *P* > 0.1).

**Testis size**

The mean testis mass in the pure subspecies differed substantially (Fig. 3A). In
hybrid males the direction of the cross had a great influence on testis mass and
the values were similar to those of the fathers' subspecies (Fig. 3A).

The relative mass of one testis, as a percentage of body mass, was very similar
in the pure subspecies (see Fig. 3B; only laboratory bred insects were dissected).
The hybrid males with *P. v. minor* mothers had a larger relative testis mass than
either of two pure subspecies (Mann-Whitney U-Test, *P* < 0.01 for both
comparisons) and the hybrid-males with a *P. v. veluchianus* mother had a smaller

![Diagram](image-url)

**Figure 3.** Mean testis-mass (±SE) of *P. v. veluchianus* males (vv), *P. v. minor* males (mm) and hybrids
males (*v*$_2$*m*$_0$: offspring of *P. v. veluchianus* $\delta \times P. v. minor$ $\varnothing$, and *m*$_2$v$_0$ for the reverse cross) measured
as absolute mass (A) (all values significantly different at *P* < 0.008 in pairwise comparisons using
Mann-Whitney with the exception of the following two pairs: vv/$v_2$m$_0$ and m$_2$v$_0$/mm) and as
percentage of body-mass (B) (all values significantly different at *P* < 0.008 in pairwise comparisons
using Mann-Whitney U-Test with the exception of vv and mm). (6 for the means between 8 and 26,
significance level *P* reduced to 0.05/6 in order to correct for the inflation of type-1 error).
relative testis mass (Fig. 3B, Mann-Whitney, \( P < 0.01 \) for both comparisons). As far as this type of hybrid is concerned there were no obvious differences between families in testis mass (Kruskal-Wallis ANOVA, values from 22 males of nine families, \( P > 0.1 \)).

**DISCUSSION**

*Inheritance of body size*

The results of the analysis of heredity of the three parameters for size showed that these characteristics differ in regard to genetic control.

Pronotum length (Fig. 1B) seems to be determined by autosomal genes because the reciprocal hybrid males had pronota of the same size. Additionally, the hybrids, males and females, were nearly as large as the insects of the nominate subspecies *P. v. veluchianus*.

Hind femur length on the other hand (Fig. 1C) seems to be partly determined by six-linked genes, since the two types of hybrid males differed in this regard. The influence of cytoplasmic or maternal effects that could cause similar effects seem improbable because the reciprocal female hybrids had the same hind femur length. These effects cannot however be excluded. Similarly, the other two body size measurements showed no indication of maternal or extrachromosomal effects.

Contrary to the other size-parameters, body mass seems to be largely determined by X-chromosomal genes (Table 2). Thus the characteristic shown to be involved in sexual selection in bushcrickets (Gwynne, 1982) is likely to be determined by hemizygous alleles in the heterogametic sex. Interestingly, other characteristics that are also assumed to be important in sexual selection, e.g. song patterns in crickets (Bentley & Hoy, 1972; Hoy, 1974) and fruitflies (Ewing, 1969), duration of larval development in crickets (probably sexually selected because of the effect on protandry, Tanaka, 1991), duration of the premating period in female noctuids (Han & Gatehouse, 1991), mate selection behaviour in female butterflies (Grula & Taylor, 1980), mate recognition in male fruitflies (Kawanishi & Watanabe, 1981) and cockroaches (Ross, 1992) and genital morphology in male fruitflies (Coyne, 1983) are all largely determined by sex-chromosomal genes in the heterogametic sex. Provided that most new mutations are recessive, the relative rate of evolution is faster for sex-linked than autosomal alleles (Charlesworth et al., 1987). Thus sexually selected genes may have a selective advantage if linked to the X-chromosome. Concluding from the above studies that the X-chromosome plays a special role in sexual selection is of course premature without also considering the negative findings as well. An extensive examination of this nature, however, would be clearly beyond the scope of this study.

The sex-linked inheritance of body mass also has important implications for the evolution of spermatophylax size, because this characteristic is closely correlated to body mass in several crickets and bushcrickets including *Poecilimon veluchianus* (Gwynne et al., 1984; Sakaluk & Smith, 1988; Wedell & Arak, 1989; Simmons & Bailey, 1990; Galliart & Shaw, 1991; Heller & Reinhold, 1994).

With sex-linked genes for body weight, the heritability calculated from the correlation between body mass or spermatophylax size of a father and its male offspring will invariably be very low. If this sex-linked inheritance of size also occurs in other orthopterans, the nonsignificant heritabilities of body mass found
in father/male offspring regressions in two crickets (Simmons, 1987; Sakaluk & Smith, 1988; Sakaluk et al., 1992) may not necessarily reflect low genetic variability for body mass.

In *Poecilimon veluchianus veluchianus* there seems to be low genetic variability for body mass as is indicated by the absence of a significant difference in the variance of body mass within and between families (Table 1). Strong sexual selection for body size occurring in bushcrickets (Gwynne, 1981, 1982) can reduce genetic variability for body size. Thus the low genetic variability in *Poecilimon veluchianus* body mass found in this study may be the result of female choice for larger males.

**Inheritance of testis size and sperm number**

The testis mass of the hybrid males also seems to be sex-linked, but resembles the values of the fathers' subspecies in each case. A sex-linked heredity from father to son, however, is very difficult to explain in species with an XO chromosome system. One mechanism that may hypothetically cause such inheritance in *Poecilimon veluchianus* is the imprinting of paternal or maternal genes. Another possibility could be the existence of a neo-XY chromosome system in *P. veluchianus* instead of the XO system which has been found in all *Poecilimon* species analysed up until now (Messina et al., 1975). A neo-XY system of this type has, for example, been found in the related bushcricket, *Barbitistes serricauda* (Messina et al., 1975). The similarity between the testis mass of *P. v. minor* males and hybrid males with a *P. v. minor* father may, alternatively, be an effect of hybrid dysfunction in these hybrids.

Contrary to the relative mass of the spermatophore gland, which differs between the two subspecies (Heller & Reinhold, 1994), relative testis mass was very similar in both subspecies. Thus the evolutionary pressures to invest energy into the growth of the testes and the spermatophore glands seems to vary with size.

Since the hybrid males inherit body mass largely from their mother and testis mass was comparable to that of the father's subspecies, the relative testis mass is bound to be very different in the two types of hybrid males. In hybrid male bushcrickets fathered by *P. v. minor*, the relative size of the testes is reduced by about 40% in comparison to the males of either pure subspecies. The reduced sperm number these hybrid males produce may reflect some threshold of testis size for optimal function since hybrid males that produced only a few sperm also had small testes (correlation coefficient: $r = 0.592$, $n = 16$, $P < 0.02$).

In crosses between animal species the heterogametic sex is more often affected by infertility than the homogametic sex. Though some theoretical reasons for this striking feature have been proposed (Zouros, 1986; Frank, 1991, but see also Coyne et al., 1991) the actual mechanisms responsible are in most cases unknown. However, the sex-chromosomes often seem to be involved (Coyne, 1992). In the subspecies hybrids of *P. veluchianus*, genes on the X-chromosome are also likely to play some major role in hybrid dysgenesis because the only genetic difference in the two types of F1-hybrid males is that one type inherits the X-chromosome from *P. v. minor* females and the other from *P. v. veluchianus* females. The other possibility, that of the incompatibility of cytoplasmic factors of *P. v. veluchianus* with autosomes of *P. v. minor* cannot, however, be excluded.
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