Growth, 1975, **39**, 251-280

A CANONICAL DISCRIMINANT ANALYSIS OF POST-EMBRYONIC DEVELOPMENT IN *NOTONECTA MACULATA* FABRICIUS (INSECTA:HETEROPTERA)

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(Received for publication March 1, 1975)

A multivariate method, canonical discriminant analysis, was used to discern a pattern of growth in *Notonecta maculata*. A stepwise procedure reduced the 11 metrical characters measured on individuals of both sexes, from the 1st larval stage to the adult, to 4 significant variates which were used in the canonical analysis. The four canonical variates accounted for the meaningful part of the variability. Relying on bivariate allometry and on an experimentation with a juvenile hormone analogue, each independent direction of variation was related to combined effects of such biological factors as a general growth trend, sex and juvenile hormone.

INDEX TERMS: Canonical analysis, discriminant, longitudinal development, juvenile hormone, *Notonecta maculata*, insecta, Heteroptera

INTRODUCTION

The biometrical study of growth was almost exclusively based upon the use of bivariate allometry (Huxley and Teissier, 1936) until the introduction of multivariate analysis. Students of growth were therefore restricted to the analysis of the changes of two body-dimensions at a time. Multivariate techniques spread with the advent of computers. These methods permit simultaneous consideration of the different measurements needed to characterize the size and the shape of a growing organism. The multivariate generalization of allometry was recently reviewed by Sprent (1972).

Since the pioneer works of Teissier (1955) it has been well known that Arthropods provide suitable material for such studies. Among the publications devoted to the subject the only studies dealing directly with insect growth are those of Blackith (1960), Blackith, Davies, and Moy (1963); Blackith and Blackith (1969).

In recent years, multivariate statistics have improved, experience has accumulated, and books (e.g., Cooley and Lohnes, 1971) have made the refinements of these techniques readily available to biol-

ogists. Nevertheless, obtaining the necessary large amount of data is still a time-consuming operation, which limits the scope of the methods, or the value of the results when the samples are too small.

The problem of size and shape variation during growth is approached here in an aquatic insect, *Notonecta maculata* Fabricius (Heteroptera). Several body dimensions were measured, for all the individuals of a sample, at each of the easily recognisable five instars of the development, and in the adult.

In a previous work (Cuzin-Roudy, 1975), the different stages of the growth of *N. maculata* have been considered separately. The within-stage variance of the data has been analyzed for each instar by a multivariate method suitable for a unique set of data: principal component analysis. Within-stage variance has also been studied, by the same method, and for the complete growth, by grouping the intra-stage dispersions into a pooled within-groups matrix.

Another analysis has been also conducted from a general point of view, considering the total dispersion (Cuzin-Roudy, in preparation). The mean deviations were then calculated from the grand mean of a unique sample grouping the different stages.

But an important piece of information is lost in this procedure because within-stages variability is not distinguished from between-stages variability. Seeking to account simultaneously for the two types of dispersion, we chose to use here canonical discriminant analysis, which maximizes between-groups variation with respect to within-groups variation. This method may be expected to give the best separation between the centroids of the stages, and, at the same time, a synthetic view of these two aspects of the variability.

In biology, canonical discriminant analysis has been employed to separate groups already known, as for example species in anthropology (Ashton, Healy, and Lipton, 1957), planctonic species (Ibanez, Ducret, and Dallot, 1974), or populations from different geographic origins (Jolicoeur, 1959; Barnes and Healy, 1969; Prunus and Lefebvre, 1971). In growth studies, the method has been used, taking the stages of the development as predetermined groups, by Blackith *et al.* (1963) in a terrestrial heteropteran: *Dysdercus fasciatus* Sign; by Blackith and Blackith (1969) on morabine grasshoppers; by Lauga (1972) on a cricket: *Acheta domesticus* L. and by Laval (1975) on an amphipod crustacean: *Phronima sedentaria* Forsk.

The data from *Notonecta maculata* analyzed here present several advantages: they deal with the same individuals measured at each stage of the development, instead of different samples for each stage: i.e., the data are longitudinal instead of cross-sectional (Tanner, 1951); both sexes are represented, and the sample size is large (50 individuals).

The post-embryonic development of *N. maculata* was prolonged by treatment with a synthetic compound having juvenile hormone activity which was applied to newly moulted 5th instar larvae. With a suitable dose of an active substance (20 µg of ester of 3,7,11-trimethyl-2,4-dodecadienoic acid per individual) a supernumerary larval instar is obtained (6th instar) instead of the imago (Cuzin-Roudy and Srihari, 1975). The 6th instar larvae show the same overall size as normal adults, but have larval body proportions. The data relating to the 6th instar individuals were introduced in the analysis for a comparison with the normal growth.

Material and Data

Notonecta maculata is a water-bug very common in France in natural and artificial pools (Poisson, 1957). The entire post-embryonic development can be followed in the laboratory. Young *Notonecta* were taken, at the first instar, from the garden basins of the "Museum d'histoire naturelle de Paris," and bred individually. The insect passes through 4 larval and one imaginal moults. Development lasted from May to July. The rigid exuviae left by each individual of the sample, at each moult, and the adults finally obtained were measured, after dissection, with a Zeiss measuring microscope. A high degree of accuracy was obtained, the error ranging from 0.4 to 1 per cent. Twelve body dimensions were measured on each individual, at each stage of its development (stages I to V, or VI) and on the adult (stage A). A total of 7,442 measurements were performed.

The characters measured were:

—the length of the three principal leg segments:

1. the anterior femur *F1*
2. the anterior tibia *Ti1*
3. the anterior tarsus *Ta1*
4. the median femur *F2*

5. the median tibia	*Ti2*
6. the median tarsus	*Ta2*
7. the hind femur	*F3*
8. the hind tibia	*Ti3*
9. the hind tarsus	*Ta3*

—other dimensions:

10. the length of the rostrum	*R*
11. the length of the hemelytra	*E*
12. the total length of the abdomen	*Abd*

A reference dimension *S*, computed as the geometric mean of the 9 leg segments:

$$S = (\log_e F1 + \log_e Ti1 + \ldots + \log_e Ta3)$$

was used in bivariate allometry.

The data utilized in the present study were used previously in a paper on the individual growth (Cuzin-Roudy, 1965) and in an analysis of the within-stage variability (Cuzin-Roudy, 1975). Within-stage means and standard-deviations are given extensively for the twelve variates in this previous work (Cuzin-Roudy, 1975, Tables 1 and 2). The reader may find here (Table 1) only the parameters concerning the 4 variates retained for the canonical analysis (see p. 258).

All the characters (except *Abd*, A) display through the whole development a very narrow distribution: within-stage coefficients of variation range from 1.89 to 4.53 per cent.

Because of the difficulties associated with the use of the hormone analogue, only a few 6th instar larvae underwent the extra larval moult, and only a limited amount of data could be gathered for the supernumerary larval instar. Their means and standard-deviations are given in Table 1.

Statistical Analysis

The mathematical principles underlying canonical discriminant analysis will not be presented in this paper. Details on the method are to be found in text-books by Rao (1952), Anderson (1958); Seal (1964); Cooley and Lohnes (1971); Blackith and Reyment (1971); Hope (1968); and Romeder (1973), among others. A useful summary

TABLE 1

MEANS AND STANDARD-DEVIATIONS (ITALICS) FOR THE FOUR VARIATES USED IN THE CANONICAL DISCRIMINANT ANALYSIS, AFTER TRANSFORMATION INTO NATURAL LOGARITHMS OF THE MEASUREMENTS EXPRESSED IN MICRONS

	Normal Growth						Extra Stage
Variates	I	II	III	IV	V	A	VI
				Males			
Ta1	6.1186	6.4240	6.7380	7.0540	7.3511	7.4965	7.5023
	0.0289	*0.0246*	*0.0243*	*0.0220*	*0.0228*	*0.0270*	*0.0271*
Ti2	6.3207	6.7778	7.1709	7.5480	7.9046	8.1384	8.1231
	0.0324	*0.0288*	*0.0302*	*0.0241*	*0.0222*	*0.0237*	*0.0301*
Ti3	6.9032	7.3002	7.6561	7.9911	8.3042	8.4909	8.5199
	0.0248	*0.0258*	*0.0276*	*0.0228*	*0.0218*	*0.0229*	*0.0336*
E	5.8235	6.2938	6.8707	7.5073	8.3530	9.3668	8.6874
	0.0386	*0.0363*	*0.0451*	*0.0408*	*0.0280*	*0.0271*	*0.0138*
				Females			
Ta1	6.1266	6.4409	6.7634	7.0937	7.3959	7.5499	7.5274
	0.0264	*0.0239*	*0.0247*	*0.0185*	*0.0207*	*0.0264*	*0.0501*
Ti2	6.3151	6.7866	7.1909	7.5816	7.9377	8.1609	8.1479
	0.0278	*0.0271*	*0.0274*	*0.0217*	*0.0219*	*0.0222*	*0.0356*
Ti3	6.9050	7.3120	7.6757	8.0280	8.3482	8.5420	8.5295
	0.0225	*0.0231*	*0.0302*	*0.0225*	*0.0212*	*0.0240*	*0.0184*
E	5.8185	6.3014	6.8869	7.5441	8.3888	9.4088	8.7108
	0.0432	*0.0312*	*0.0450*	*0.0323*	*0.0226*	*0.0236*	*0.0282*

appears also in a recent paper by Phillips, Campbell, and Wilson (1973: 40-45). Due to uncertainties in the vocabulary, it seems better to specify that we mean by "canonical discriminant analysis" the solution of the matrix equation:

$$(\mathbf{B} - \mu_j \mathbf{W})\mathbf{c}_j = 0$$

where $\mathbf{c}_j$ stands for the jth canonical vector, corresponding to the canonical root μ_j. **B** is the matrix of between groups sums of squares and cross-products; **W**, the matrix of pooled within-groups sums of squares and cross-products. In practice, only the first few canonical roots may be needed to explain the major part of the total variation.

We followed Cooley and Lohnes (1971) for the theory of the method, though we wrote a different FORTRAN program, better suited to the handling and final representation of our data than the DISCRM program of these authors. A checkout done with the DISCRM program showed no divergences in the common results.

Contrary to the DISCRM program procedure, we chose to standardize the canonical vectors and to compute the "structure coefficients" in relation to the pooled-within-groups variance-covariance matrix, rather than to the total dispersion. This procedure is mentioned by Cooley and Lohnes (1971: 250) and illustrated by Porebski (1966). Then, in place of the total variance, the average within-groups variance is set to unity. In this case the structure coefficients correspond to within-groups correlations between the original variates and the individual discriminant scores of each group. In fact an average value is obtained for all groups. Standardization in relation to the total dispersion matrix, which is more usual, would lead to correlations between the original variates and the discriminant scores of the individuals arranged in a unique set, without distinction between the development stages. This last procedure seems to be of lesser interest for a growth study, because the correlations with the first canonical axis would then be all near unity, and very weak with regard to the following axes. More is to be learned with within-groups variation. Besides, setting to unity the average within-groups variance has the additional advantage of permitting an a posteriori check of the homogeneity of the group dispersions (Hope, 1968: 117).

The 12 characters measured are strongly correlated and the question arose if a reduced number of variates would be sufficient to account for the main directions of variability. To reduce the number of variates, the stepwise procedure in the MAHAL 3 program of Romeder (1973) was used. At each step, the variate maximizing the trace of $\mathbf{T}^{-1}\,\mathbf{B}$ is selected, a criterion which constitutes a generalization of the Mahalanobis' D^2 (Romeder, 1973: 77); $\mathbf{T}$ stands here for the total sums of squares and cross-products matrix. The MAHAL 3 program also gives the user the opportunity to employ a "test-sample," a procedure not carried out in a preceding study (Laval, 1975), owing to the small number of data. The concept underlying the "test-sample method" (Romeder, 1973: 83-99) is to select, prior to the analysis, a random sub-sample among the individuals of each group (usually a number proportional to the group size). The computation is first run on the "basic-sample" constituted by the remaining individuals. Once the discriminant functions are established, they are applied to the test-samples, which allows an evaluation of the percentage of correct classification, based on samples coming of course from the same pop-

ulations. This empirical test is of practical use in determining the moment at which the addition of new variates ceases to improve the value of the discrimination.

The MAHAL 3 program executes a brief canonical discriminant analysis at each step when the percentage of correct classification falls down. We used this program merely for its stepwise procedure.

The other computer programs used in this study are: the A3.1 program of Sokal and Rohlf (1969) to verify the normality of the variates; the MANOVA program of Cooley and Lohnes (1971) to test the equality of group dispersions; the NUAGES program (Laval, 1974) for three-dimensional representations. The computations were performed on the IBM 7040 and 360/65 computers of the Nice and Meudon Observatories.

Data Transformation

In a growth study, it is advantageous to transform the data into logarithms, to obtain linear relations between measured characters (Jolicoeur, 1963). The logarithmic transformation results also in a better normality of the data distributions and homogeneity of the dispersions, as already observed in a previous study with the same data (Cuzin-Roudy, 1975). All the computations reported here are thus based on the data transformed into (natural) logarithms. It should be noticed that Blackith *et al.* (1963); Blackith and Blackith (1969); and Lauga (1972) have done their computations on raw data, leading to different results.

Normality of the Transformed Data Distributions

The mathematical methods used throughout this work assume a multivariate normal distribution of the characters. Normality of marginal distributions does not guarantee that the whole distribution is multivariate normal (Cooley and Lohnes, 1971: 38) but nevertheless gives a strong presumption that multinormality is respected. For each transformed variate, normality was tested, at each stage, using Kolmogorov-Smirnov's D and g_1 and g_2 statistics given by the A3.1 program (Sokal and Rohlf, 1969). All the D values were found to lie below the 1 per cent probability level. The D value for *Abd* at stage A ♂ is significant at the 5 per cent level. The corresponding value of g_1 is positive and also significant at the 5 per cent level. Looking at the

histogram of *Abd* A ♂ (not shown), it becomes apparent that the distribution is trimodal. Going back to the individuals of the sample, it was found that the abdomen of adults could be encountered in three states of extension, depending on the conditions of fixation of the specimens: the genitalia could be retracted, extended or in an intermediary state. The same thing occurred in females, leading to a *D* value just below the 5 per cent level. We came to the conclusion that it would be better to discard the measurements of the variate *Abd* from the whole analysis, even if its distribution is fairly normal at the larval stages.

Stepwise Discriminant Analysis

The MAHAL 3 program (Romeder, 1973) was run with 12 groups (6 male and 6 female stages), and with the 11 variates remaining after the elimination of *Abd*. For each group, the 50 measured individuals were divided into a test-sample of 10 specimens selected at random, and a basic-sample formed with the remaining 40 individuals. Fig. 1B displays the results of the stepwise selection of the variates. The percentages of correct classification for the basic- and test-samples are plotted against the step numbers, together with the selected variates. The first character drawn out is the hemelytron *E*. This result is not surprising, because this measure is the one which shows the greatest increases between stages, permitting thus the best discrimination.

At step no. 1, the percentages of correct classification are respectively 66 and 59 per cent for both the basic- and the test-sample. Looking back at the detailed classification tables given by the program output (not shown), one can see that the 40 per cent misclassifications are only due to a confusion between males and females at a given stage. Univariates tests for the intra-stage differences between means show indeed that this character does not differ much among sexes (Cuzin-Roudy, 1975), especially at the beginning of the development. When the percentages are computed ignoring the sexes, 100 per cent of correct classification are obtained from the first step. For both samples (Fig. 1A), each individual is allocated to its proper stage. The percentage of correct classifications remains at a high level until step no. 5, where it falls down for the test-sample (Fig. 1A). This means that several errors of stage allocation occur when the discrimi-

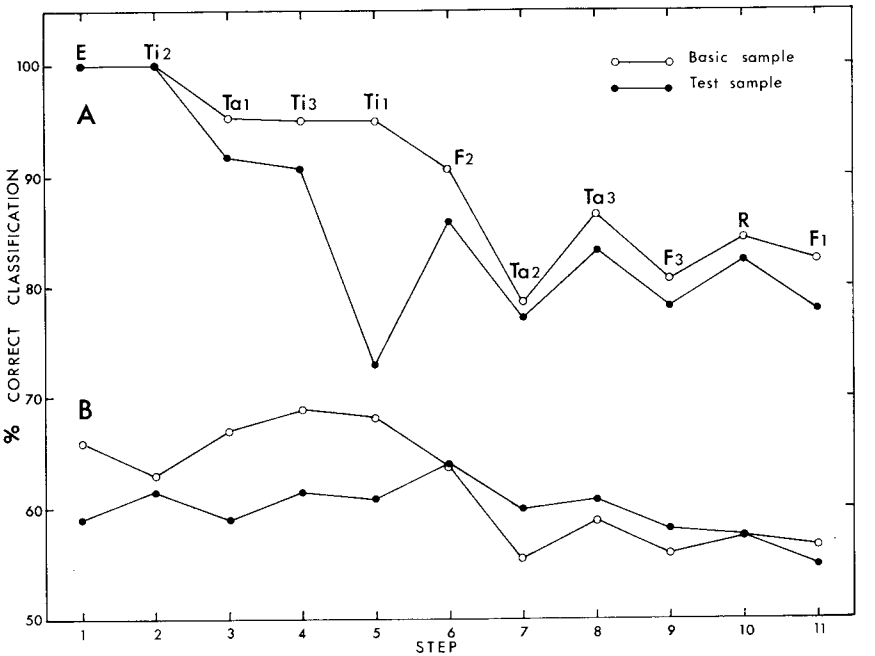

FIGURE 1

Stepwise discriminant analysis. Evolution of the percentage of correct classification at each step, for the basic- and the test-sample B: the sexes being considered separately; A: the sexes being considered together.

nant functions are applied to this sub-sample, showing that the addition of new variates does not improve the discrimination any more. At step no. 6 the percentage of the basic-sample itself falls down.

When percentages are computed considering males and females as different groups, Fig. 1B shows a fall at step no. 5, accentuating after step no. 6.

It is obvious that, by step no. 6, there is no gain in discrimination when new variates are added. Even at step no. 5, the separation seems to be illusory, as indicated by the decreasing percentage of the test-sample. A comparison with the results of a canonical analysis realized with the 11 variates, shows that the 5th variate (*Ti1*) shares with the 4th (*Ti3*) the correlations with the axes, and does not bring any new elements. The discriminant analyses (done by the MAHAL

3 program at each step where the percentage falls down) show that the configuration of the groups (but not the position of the individuals) remains the same from step no. 4. With the 11 variates, the group configuration does not differ essentially from the one obtained with the first 4 selected variates. Thus we will only retain these 4 characters (*E, Ti2, Ta1* and *Ti3*) sufficient to give an accurate picture of the relations between the stages, while being easier to interpret. Moreover, the accuracy of the computations is increased when matrices to be inverted are of lower order.

Homogeneity of Within-Group Dispersions

Homogeneity of group dispersions is theoretically a pre-requisite for a discriminant canonical analysis. An appropriate test of significance is provided in the MANOVA program (Cooley and Lohnes, 1971). We obtained for the four variates and the twelve groups a highly significant F-value of 2.17 (for 110 and 312,792 D.F.; $F_{0.05} = 1.31$; $F_{0.01} = 1.46$). The within-group matrices cannot thus be strictly considered as homogeneous. However, it should be emphasized that this test is very sensitive to deviations from strict multinormality (Hope, 1968).

On the other hand, it was found, after completion of the analysis, that the standard-deviations of the discriminant scores (for each group, on each axis) were not very different from unity (they ranged from 0.64 to 1.39). This indicates a posteriori that the original group dispersions were not too heterogeneous (Hope, 1968: 117). As a matter of fact, canonical discriminant analysis, as well as the other methods of multivariate analysis, is a powerful descriptive tool, even if the conditions of normality or homogeneity are not strictly met: the discriminant functions are in any case those giving the best discrimination between the group centroids. For an overall view of the growth process, we are interested with the interrelations between the developmental stages rather than with differences the reality of which is obvious. Except in some critical situations where a test is actually needed, we will be content with knowing the relative position of the groups, their relations with the axes, and the variates involved. Following in this matter Blackith and Reyment (1971) we will not pay too much attention to the exact application of the statistical tests,

but instead we will pursue our analysis as long as the results are biologically meaningful.

Canonical Discriminant Analysis

Table 2 reports the main numerical results of the canonical analysis run with the 4 variates selected by the stepwise procedure. Males and females were introduced into the analysis as different groups.

In a canonical discriminant analysis the maximum number of discriminant functions which can be found is the smaller of (1) the number of variates and (2) one less than the number of groups. Thus in the present case a limit of 4 canonical axes may result from the analysis.

On a study where the principal direction of variation is due to the simultaneous increment of all the variates, it is not surprising that the first canonical variate accounts for a very important part of the total variation (95.4 per cent). The remaining 4.6 per cent represent independent sources of variability and may be more instructive. The 0.01 per cent of the total accounted for by the 4th axis may appear negligible; nevertheless Bartlett's test (though some reservations can be made about its strict conditions of application, see p. 260) gives a chi^2-value still highly significant for the 4th canonical root: 77.80 with 8 D.F. ($chi^2_{0.05} = 15.51$; $chi^2_{0.01} = 20.09$). The corresponding canonical correlation (cf. Cooley and Lohnes, 1971: 249), $R = \sqrt{\mu_4/(1 + \mu_4)}$, amounts to 0.351. So we will still search for a biological interpretation for that axis.

The structure coefficients, i.e., the correlations between the vari-

TABLE 2

Canonical Discriminant Analysis (4 Variates, 12 Groups). Elements of the Canonical Vectors (Standardized in Relation to the Pooled-Within Groups Dispersion Matrix); Canonical Roots μ_j, and Corresponding Percentages of the Total Variance

Canonimal Axes	Variates				μ_j	% of Total Variance
	E	*Ti2*	*Tal*	*Ti3*		
Axis I	25.358	4.778	—2.738	4.092	1231.40	95.41
Axis II	25.659	—25.212	—6.800	—22.240	57.35	4.44
Axis III	—0.815	—50.169	59.328	5.914	1.79	0.14
Axis IV	1.035	—48.571	—23.480	73.447	0.14	0.01

TABLE 3
STRUCTURE COEFFICIENTS, COMPUTED FROM THE CANONICAL VECTORS, STANDARDIZED IN RELATION TO:
—the pooled-within-groups dispersion matrix.
—the total dispersion matrix (italics).

Canonical Axes	Variates			
	E	*Ti2*	*Ta1*	*Ti3*
Axis I	0.990 *1.000*	0.688 *0.978*	0.577 *0.979*	0.665 *0.977*
Axis II	0.126 *0.028*	—0.674 *—0.209*	—0.542 *—0.200*	—0.667 *—0.213*
Axis III	0.052 *0.002*	—0.137 *—0.009*	0.561 *0.045*	0.083 *0.006*
Axis IV	—0.021 *—0.001*	—0.231 *—0.010*	—0.242 *—0.012*	0.326 *0.015*

ates and the canonical axes, are displayed in Table 3. We have seen earlier that it was possible to standardize the canonical vectors in relation (1) to the pooled-within-groups dispersion matrix, and (2) to the total dispersion matrix. Both analyses were run, and the different resulting structure coefficients are shown in Table 3. It is clear that the second method (Table 3, italics) is of minor interest in this study. All the correlations with the 1st axis are so high that those with the remaining axes appear negligible. This is due to the very small within-groups variation relative to the total variation, as it is apparent from Table 4. Size increase in *N. maculata* is indeed very important between successive stages, while within-groups variance is very low (Table 1). The standardization of the canonical vectors in relation to the pooled-within-groups dispersion emphasizes this within-stages variation, without obliterating the variation due to growth. From the structure coefficients, it is then possible to evaluate the percentage of the within variance expressed by each canonical axis, by dividing the sum of squares of the coefficients for one axis by the trace of the correlation matrix **R** based on **W** (cf. Cooley and Lohnes, 1971: 253). When this is done, the first canonical axis is found to represent only 55.7 per cent of the whole pooled-within-groups variance; the second 30.3 per cent, the third 8.6 per cent and the fourth still 5.5 per cent. At this stage it becomes clear that we should base

TABLE 4
POOLED-WITHIN-GROUPS MATRIX W (ABOVE DIAGONAL) AND TOTAL DEVIATION MATRIX T (BELOW DIAGONAL) OF THE SUMS OF SQUARES AND CROSS-PRODUCTS

W / T	E	Ti2	Ta1	Ti3
E	0.73923 / 889.67	0.32623	0.27652	0.28770
Ti2	451.59	0.40353 / 242.77	0.28110	0.30362
Ta1	354.34	189.98	0.35327 / 149.14	0.24783
Ti3	399.38	214.87	168.27	0.33862 / 190.33

our interpretation instead upon the structure coefficients computed in relation to the pooled-within-groups dispersion.

All the correlations with the first axis are positive and high for the 4 variates, principally for *E*, the character showing the greatest increases.

The second canonical variate results from a contrast between the leg segments (*Ta1, Ti2 and Ti3*) and the hemelytron *E*.

Ta1 contrasts with *Ti2* on the third canonical axis. Coefficients for *E* and *Ti3* are almost zero.

E is also near zero on the fourth axis, which opposed *Ti3* on the one hand, and *Ta1-Ti2* on the other.

Applying the canonical discriminant functions resulting from the analysis (Table 2) to the original variates gives the discriminant scores on the 4 axes for the individuals, computed as:

$$y_j = c_{ij}(X_i - \overline{X}_i) + \ldots + c_{pj}(X_p - \overline{X}_p)$$

where y_j = individual score on the jth axis;
c_{ij} = element of the jth canonical vector corresponding to the variate X_i ($i = 1, p$), expressed as a deviation from the stage grand mean $\overline{X}_i$.

Groups centroids are computed in a similar way using the stage means.

The individual points cannot be plotted simultaneously in relation to the 4 axes. It is still possible to represent a three-dimensional space as a perspective view in two dimensions; the NUAGES program (Laval, 1974) was used to display an overall view of the centroids in relation to the first 3 axes (Fig. 2). For a more detailed interpretation, it is however more instructive to examine the axes two at a time.

Figure 3 shows a representation of the centroids and of the individuals in the plane of the first two canonical variates. It may be observed that instars are ranked in order from the first stage to the adult on the first axis, a situation arising from the simultaneous increase of the characters. Thus the first axis retains the chronology of the development, and gives a yardstick for subsequent comparisons with the other axes. Looking at the reference dimension *S*, it may be verified that within a defined stage the individuals of each group (♂ or ♀) are ranked along the first axis according to their size. While this is somewhat obscured by the different scales for the axes in Figure 3, it is also to be observed that the centroids of females (becoming, in the average, larger than the males, at the end of the development) are displaced towards the positive pole of the first axis in the last stages. It would be interesting to know at what stage they can be considered as distinct from the male centroids. Unfortunately, no statistical tests are available for the comparison of two group means out of several, along a particular canonical axis. Following Blackith and Reyment (1971: 40), we conducted an analysis of variance between the scores of males and females, for each stage. Prior to this analysis, normality of scores distributions has been checked out with Kolmogorov-Smirnov's test and $g_1 - g_2$ statistics. The normality appeared to be always correct: the *D*-values never reach the 5 per cent probability level, and g_1 and g_2 never show skewness nor kurtosis. The analyses of variance reveal that sexes differ significantly (at the 5 per cent level) from stage III, and highly significantly (at the 1 per cent level) from the stage IV onwards. These

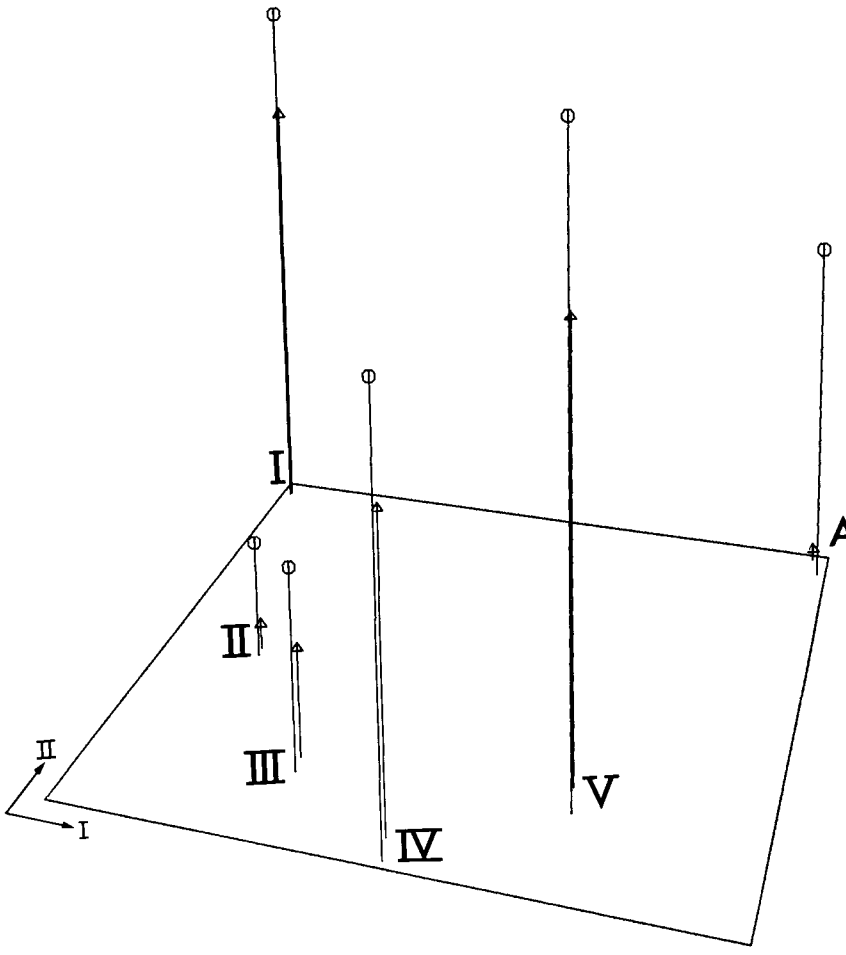

FIGURE 2
Perspective view of the stage centroids in the space of the first three canonical axes, describing the growth of *Notonecta maculata*. The axes are not to the same scale. Triangles: males; circles: females.

results were already suggested by *t*-tests made on the marginal distributions of the variates.

In summary, the first axis corresponds (1) to the increase of size resulting from growth, (2) to the within-stage variability, and (3) to some degree, to the sexual difference, acting on size.

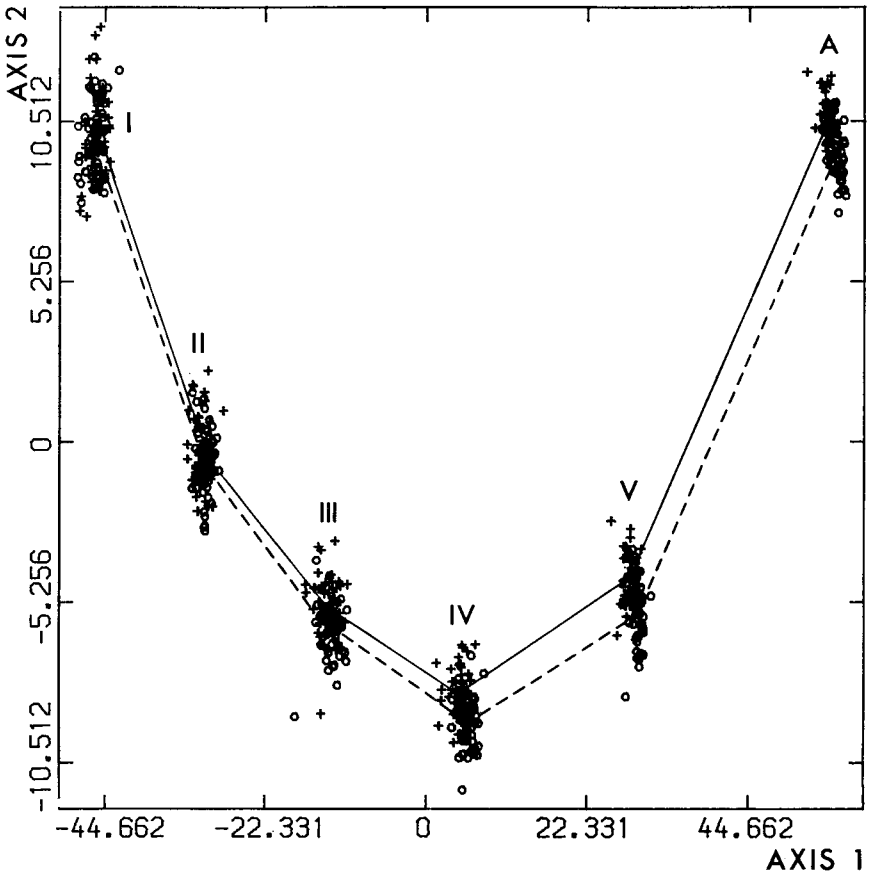

FIGURE 3

Canonical discriminant analysis: plane of the axes I and II. Circles and crosses show respectively the position of the 50 male or female individuals, at the 6 stages of the growth of *Notonecta maculata*. Group centroids are joined by a continuous line for the males, and a broken line for the females. The scale of the second axis is expanded relative to the first.

Along the second canonical variate, centroids scores decrease until the stage IV and then increase for stages V and A. This results in a "parabolic" disposition of the stages into the plane of axes I and II. As is apparent from inspection of the structure coefficients (Table 3), the second canonical variate results from a contrast between the hem-

elytra on the one hand and the three leg segments on the other. The canonical analysis run with the 11 variates (not presented here in details) shows that E is opposed to all the leg segments on the second axis. Since the reference dimension S is computed from the sum of the 9 leg segments, this opposition resolves itself into a simple case of bivariate allometry between E and S. The growth of E in relation to S can then be worked out using the classical methods of allometry.

Figure 4A displays the relations between the stage means of E and S for the males, plotted as usual in double logarithmic scale; a similar figure would be obtained for the females. The fitted curve is the "reduced major axis" (Teissier, 1948), very close here to the linear regression line. From this figure, it is apparent that the relative growth of E does not strictly follow the classical linear allometric relationship (Huxley and Teissier, 1936), as was the case for all the other characters (Cuzin-Roudy, 1965). The means of stages I and A lay above the line, while the other stage means are placed below. This becomes more apparent when the deviations from the straight line ($Y_{observed} - Y_{estimated}$) are plotted against S for each stage (Fig. 4B), according to a technique similar to the one proposed by Richards and Kavanagh (1945). The "parabolic" arrangement of the stages in the plane of the canonical axes I and II (Fig. 3) is thus found again in the bivariate case.

Not obtaining a linear disposition with a double logarithmic plot means that the ratios of E and S growth rates are not constant with time. The growth rate of E actually increases at each moult, especially at the end of the development (from 1.60 at the first moult to 2.76 at the imaginal moult), while those of the other characters remain constant, or even decrease at the last moult.

It is also possible to fit a polynomial regression of degree 2 to the curvilinear relation (in log-log) between E and S. The part of the total variance explained by the regression is then higher (99.3 per cent instead of 95.2 per cent), but the choice of such a polynomial curve is arbitrary.

On the other hand, a semi-logarithmic transformation (E in logarithms and S in millimeters) appears effective for restoring a simpler straight line relationship. The points corresponding to the stage means are now very close to the line (Fig. 4C). The part of the variance explained by the linear regression is now 99.6 per cent. This linearity

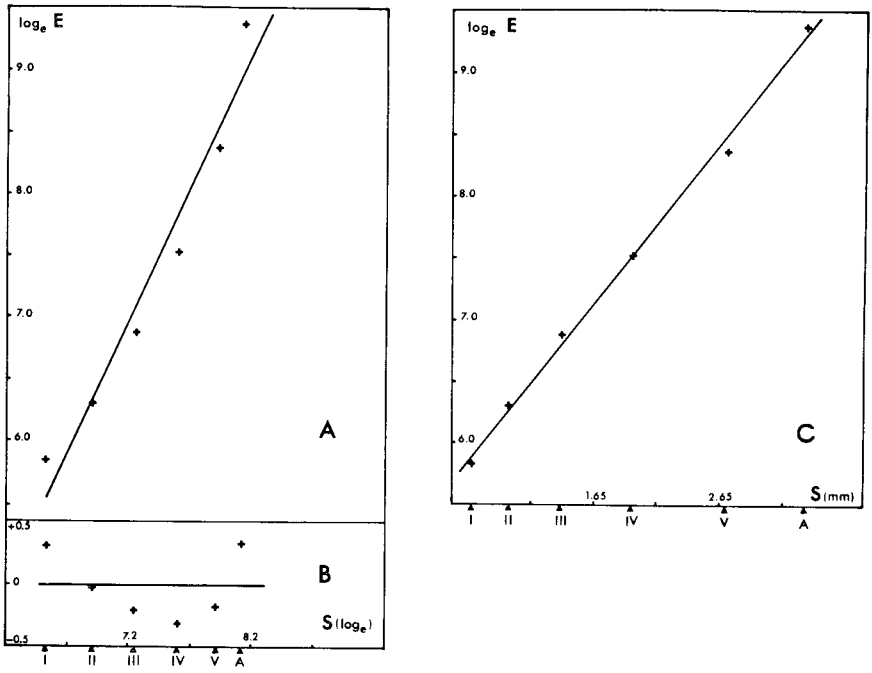

FIGURE 4

Relative growth of the hemelytra E plotted in relation to the reference dimension S, for the males.

A. In double logarithmic coordinates with the "reduced major axis" fitted to the stage means.

B. Deviations ($Y_{observed} - Y_{estimated}$) from the allometric relation (A), plotted against S.

C. In semi-logarithmic coordinates (E in logarithms and S in millimeters); the line fitted is the "reduced major axis."

in semi-logarithmic coordinates is the mark of an exponential relation of the form:

$$y = Be^{ax}$$

between the non-transformed variates E (corresponding to y) and S (corresponding to x). In natural logarithms this equation becomes:

$$\log_e y = \log_e B + ax$$

$\log_e B$ being the constant, and a the slope of the straight line. At each

moult, the slope represents the ratio of the geometric increase of E to the arithmetic increase of S. The hemelytra thus follow quite a different law of growth from the leg segments. The "parabolic" arrangement of the stages in the plane of the axes I and II is directly related to this particular way of growth of E. This variate increases along with the other measured characters, but with a higher rate (as shown by its strong correlation with axis I), according to a different mode, which finds its expression on the second canonical variate.

A high titer of juvenile hormone can be artificially re-established, at the beginning of the 5th instar, when the natural hormone disappears from the haemolymph of the insect. The imaginal moult is then inhibited, and a supernumerary larval instar (stage VI) is obtained (Cuzin-Roudy and Srihari, 1975). Only 11 perfect 6th instar larvae (7 ♂ and 4 ♀) were at hand at the end of the experiment, owing to treatment difficulties. The canonical analysis was re-run including these individuals at a supplementary stage. Figure 5 shows that stage VI is not on the trajectory of the "parabola," but outside, near stage V. The stage VI discriminant scores on the first canonical variate are intermediate between those of stages V and adult; on the second canonical variate, they are, in the average, lower than those of stage V.

This position may be explained as a result of an inhibitory effect of the juvenile hormone analogue not only on the morphogenesis of E, but also on its growth rate. At the supernumerary moult, the leg segments present a rate of increase similar to the one of the imaginal moult (Table 1), whereas E increases at a very reduced rate (1.42 instead of 2.76, a value even lower than the larval rates). This low increase of E leads to a smaller value, on the first axis, for the overall size (in which E acts together with the leg segments), and for the ratio E/leg segments, which governs the position on the second axis.

On the second axis, males and females are separated more and more as development proceeds. This is confirmed by analyses of variance: there is a highly significant separation between the scores of the two sexes from stage III (Table 5). The normality of the score distributions was verified by the Kolmogorov-Smirnov's test prior to the analyses. Looking at the measurements of the extreme individuals, it was also confirmed that the hemelytra tend to become longer (in relation to the leg segments) in males than in females.

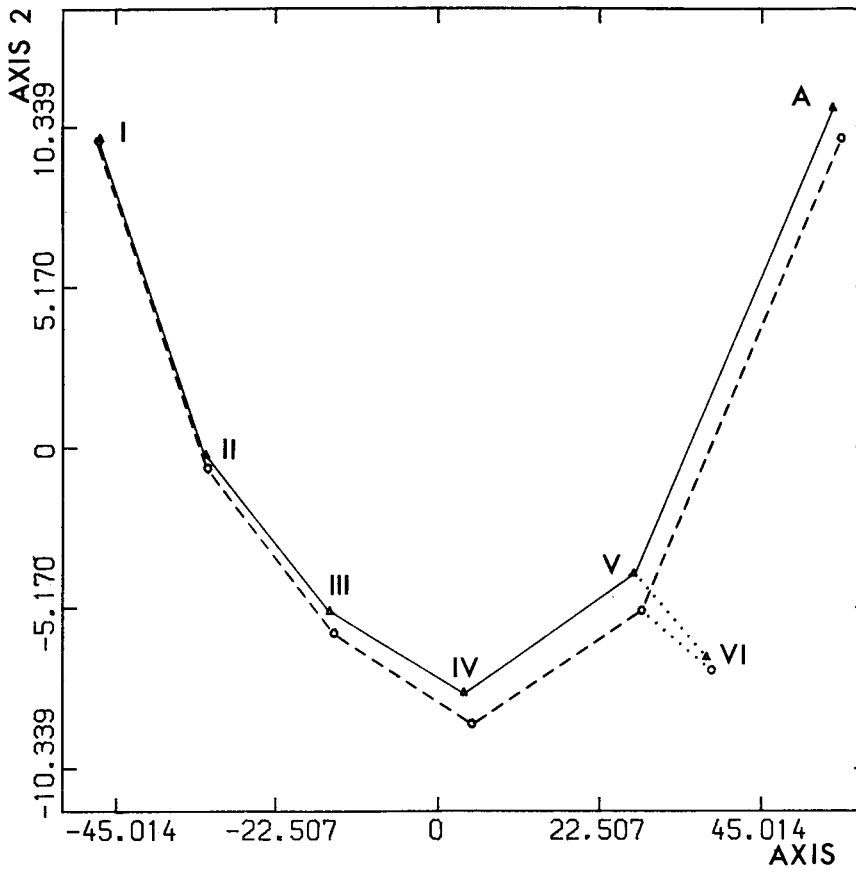

FIGURE 5
Canonical discriminant analysis of the prolonged growth of *Notonecta maculata:* plane of the axes I and II. Position of the supernumerary stage centroid (VI) in relation to the normal growth.

Figure 6 displays the position of the individuals and of the centroids in the plane of axes I and III. It can be seen that the stages are arranged along a broken line, stages II and A being at the minimum and stages I and V at the maximum on the third axis. The third canonical variate opposes *Ta1* and *Ti2,* the two other characters having structure coefficients close to zero (Table 3). We are again lead to a bivariate situation. Looking at the allometric relation of *Ta1* against

TABLE 5
ANALYSIS OF VARIANCE BETWEEN MALE AND FEMALE DISCRIMINANT SCORES ON THE FOUR CANONICAL AXES, FOR EACH DEVELOPMENTAL STAGE
$F_{0.05[1,98]} = 3.94$ and $F_{0.01[1,98]} = 6.90$

Canonical Axes	Stages					
	I	II	III	IV	V	A
Axis I	0.54	1.48	4.10*	29.88**	59.98**	68.79**
Axis II	0.10	3.55	13.22**	36.26**	41.33**	36.49**
Axis III	8.02**	10.00**	14.49**	26.76**	48.84**	121.81**
Axis IV	0.75	0.06	0.47	1.11	12.45**	46.07**

Ti2 (not presented here) it may be observed that the position of the centroids in the plane of axes I and III reproduces, with a very high magnification, the very small deviations from linearity (the linear correlation between *Ta1* and *Ti2* stage means amounts to 0.999).

Turning back to the original measurements, it is easily confirmed that the individuals which lay closer to the positive extremity of the third axis show a higher *Ta1/Ti2* ratio, the reverse being true for the opposite extremity.

Figure 6 also makes a remarkable feature clear: from the beginning of the development, the sexes are very distinct on the third axis. Females stand near the positive pole and present, in the average, a *Ta1/Ti2* ratio higher than males. Using analyses of variance between the sexes scores, in the same way as for the other axes (the normality of the distributions first being tested) we found that the separation is effective for every stage (Table 5). Thus, considering the four original variates, the *Ta1/Ti2* ratio is the best criterion for sex distinction. The third canonical variate is thus related to sex which is expressed in the relative proportions of *Ta1* and *Ti2*.

Figure 7 represents the plane of the 1st and the 4th axes. The stages are now placed in an almost linear fashion, expressing the linearity of the growth relations between the variates correlated with the fourth axis (*Ti3, Ta1* and *Ti2*). On this axis, the individuals are ranked according to their proportions between *Ti3* on the one hand and *Ta1* and *Ti2* on the other. Individuals with a relatively larger *Ti3* are closer to the positive extremity of the 4th axis.

The two sexes appear to be distinct on the 4th axis, at stages V and A. As was done for the other axes, analyses of variance show that there is a highly significant separation between sexes for the two last

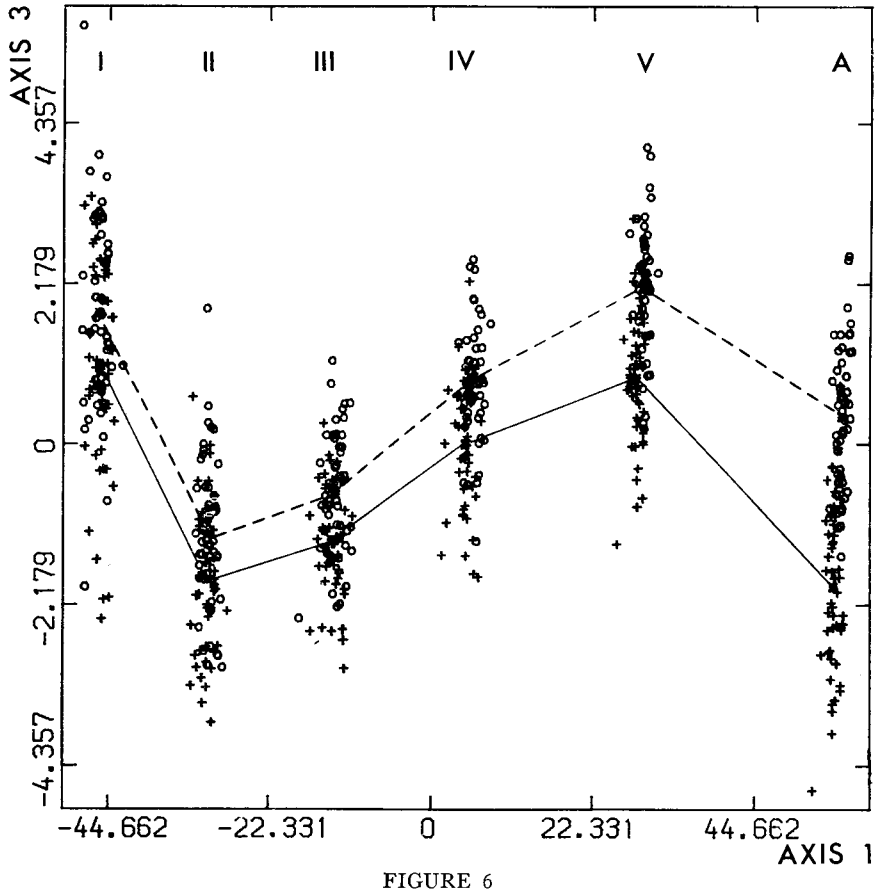

FIGURE 6
Canonical discriminant analysis: plane of the axes I and III. Legend as in Figure 3.

stages, and a nonsignificant one for the others (Table 5). Normality was always correct.

At stages V and A, the balance between the proportions of *Ti3* and *Ta1-Ti2* is thus different for the two sexes. Females have a higher *Ti3/Ta1-Ti2* ratio.

Discussion

Before discussing the results it seems worth turning back to the conditions (multinormality, homogeneity of the variance-covariance

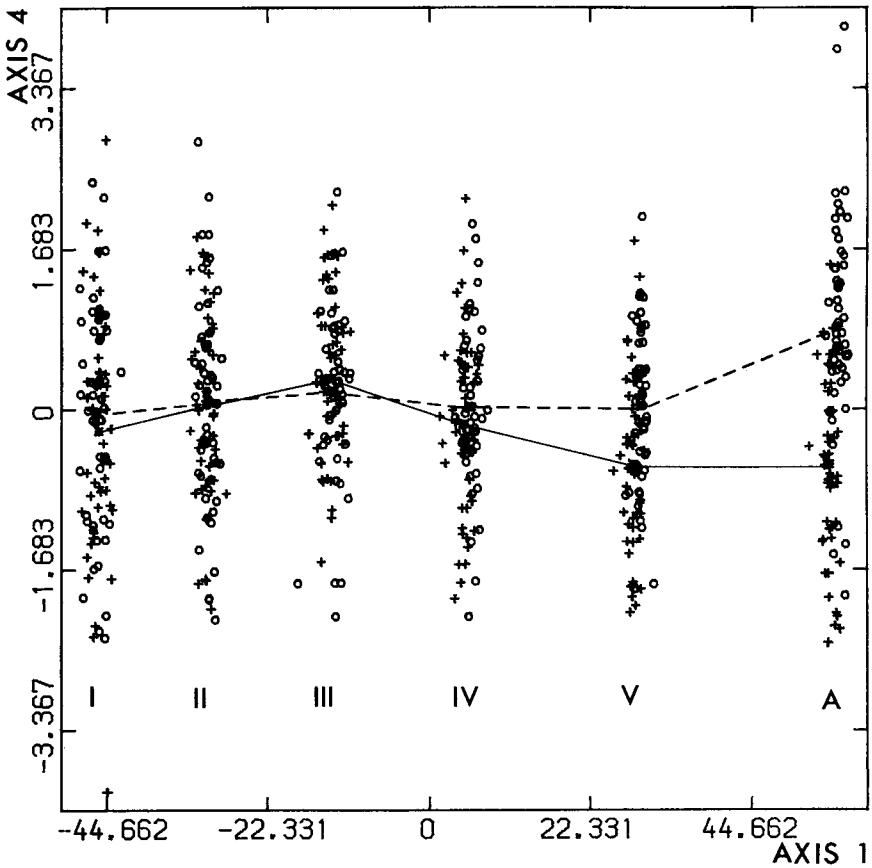

FIGURE 7
Canonical discriminant analysis: plane of the axes I and IV. Legend as in Figure 3.

matrices) required by the discriminant canonical analysis. These assumptions are seldom respected in the biological applications of the method, a fact which tends to invalidate the results obtained.

The data used here do not deviate from the required conditions to the point of introducing a noticeable bias. The large number of measurements, their accuracy, the small intra-stage variability, the presence in the analysis of both sexes, give statistical stability to the results and allow a more exhaustive interpretation. But the adequacy

of canonical analysis for such a study of growth, where the measurements are repeated on the same specimens at each stage, and are thus correlated in time, remains questionable and may be argued (Kowalski and Guire, 1974). The method does not take into account this peculiarity and treats the different groups as independent samples. However, it appears to us that this way of proceeding results in a loss of information about individual growth, rather than in erroneous assertions.

Our approach to the problem being more descriptive than theoretical, we consider that the results obtained warrant attention to and justify the use of the method in the present study.

Rather than taking into account only the first axes of an analysis conducted on 11 variates, we employed the stepwise procedure to select the most discriminant characters. This preselection of the variates gives the advantage of focusing the analysis on the significant part of the variability, discarding the redundant variates. The number of axes to be extracted is thus reduced before the start of the analysis. All of them are capable of contributing to the discrimination and of leading to a biological interpretation.

The first canonical axis accounts for 95.4 per cent of the total variance, a result which contrasts with those obtained by other authors who used discriminant canonical analysis in growth studies. Blackith *et al.* (1963) found 65 per cent for the first axis, in the heteropteran *Dysdercus fasciatus;* Blackith and Blackith (1969), 86.7 per cent in morabine Orthoptera; Lauga (1972), 84 per cent for the last stages of the development of *Grillus;* and Laval (1975) 80 per cent for the post-larval growth of the amphipod *Phronima.* It has to be noticed that these authors (except Laval) have worked on data not transformed into logarithms. The within-stage variance was then, in these studies, increasing from the first to the last stage, at the same time as the dimensions of the characters. Therefore the pooled-within-groups dispersion matrix was calculated from nonhomogeneous within-stage matrices, which makes the application of the method questionable. Moreover, the cited authors do not give details of the amplitude of the within-stage variance of their material. This dispersion was probably large compared to the total- and the between-stages variance. In such cases, an important part of the total variance is thus distributed on axes other than the first, even when the relations between the

variates are multilinear. The relatively weak percentage (80 per cent) found by Laval (1975) for the first axis, with transformed data, corresponded indeed to a large within-stage variability in *Phronima*. On the contrary, in *Notonecta,* the within-stage variability is so small that between-stages variance becomes practically equivalent to total variance (Table 4). The results become eventually similar, for the distribution of the variance on the axis, to those which would be obtained from a principal component analysis of the total variance. This explains the very high percentage of variance taken by the first axis in the present study.

A similar "parabolic" disposition of the stages in the plane of the first and second axes has already been obtained by Laval (1975, Fig. 14) for the growth of the males, in *Phronima*. Blackith *et al.* (1963, Fig. 2) did not observe this pattern for *Dysdercus* growth. On the contrary, stages I to V ranked in order along the second axis. A curvature is observed for the pattern of growth in Morabinae (Blackith and Blackith, 1969, Fig. 4). But these two patterns were obtained from raw data, which renders the comparison difficult.

The origin of the "parabolic" pattern could be imputed, in *Notonecta,* to the particular growth of the variate *E,* which results in non-linear relations with the other measured characters. The same interpretation holds for *Phronima:* the character the most connected with the second axis (*lPl1:* width of the first pleopod peduncle) is also the one having a non-linear relation with the other studied characters (Laval, unpublished). It seems probable that these observed deviations from simple allometry can be attributed to the action of factors operating specifically on these characters.

In *Notonecta* we demonstrated that the "parabolic" course of development in the plane of the first two axes, is broken when the insects are treated at the 5th instar with a juvenile hormone analogue (Fig. 5). The development of *E* (morphogenesis and growth) is actually inhibited, like the development of the other imaginal characters, by the hormone (Williams, 1961). It is tempting to attribute the special mode of quantitative growth of *E* to an influence of the variations of the juvenile hormone titer during larval development. Novak (1954) has shown in 18 insects species that the corpora allata, the hormone secreting organs, have a negative growth allometry in relation to body-length or other linear dimensions (head width, length

of the tibiae, etc.). If, as this author assumes, the hormone production is proportional to the gland size, then the hormone titer diminishes in the haemolymph while development progresses. We have seen here that the growth rate of *E* progressively increases from one stage to the next, and thus appears to be inversely proportional to the amount of inhibitory hormone present in the haemolymph of the insect. At the imaginal molt, juvenile hormone disappears, inhibition is cancelled and *E* undergoes at once its final morphogenesis and its maximum increment. The exponential mode of growth of *E*, relatively to body-size (Fig. 4) would thus reflect the sensibility of this character to juvenile hormone diminishing titer.

In *Phronima,* the character *lPl1* is obviously a sexual variant, the strong enlargement of the pleopod peduncles being a male characteristic. Charniaux-Cotton (1957) has shown, in another amphipod crustacean: *Orchestia gammarella,* that growth of sexual variants is dependent upon the androgenic hormone. Looking at the graph displaying the relative growth of three sexual variants of this species (Charniaux-Cotton, 1957, Graph 2) it can be easily verified that the points corresponding to the 2nd gnathopod propodite (*Pgn2*) for the prepuberal phase, describe a curve of parabolic aspect. If it is useful for an accurate description of this complex growth to subdivide the curve into three successive straight lines ("étapes indifférenciée, juvénile, intermédiaire"), a progressive acceleration of the growth of *Pgn2* is nevertheless evident. The great frequency of the moults, associated with an important individual variability in this species, makes clear all the intermediate steps of this acceleration. At the "post-pubérale" phase, when the hormone has no effect any more, an allometric equilibrium is recovered. In the same graph, the representative curve of growth of the 7th pereiopod (*Mp7*), an other sexual variant, is displaced relatively to the *Pgn2* curve, because of a postponed response to the hormone. The character *Cgn2* (2nd gnathopod carpopodite) responds simultaneously with *Mp7* to the hormone, but in an opposite way.

In all the preceding examples, a curvilinear relation between a character and body-size is thus an indication of a specific sensitiveness to an hormonal factor that exerts an action on quantitative growth: the androgenic hormone in *Orchestia,* and very probably in *Phronima,* the juvenile hormone in *Notonecta.*

The larger the part of the total variability controlled by these particular growth relations, the better canonical analysis succeeds in displaying them in evidence, and the more obvious is the "parabolic" aspect of the growth pattern in the plane of axes I and II.

Sexual dimorphism is distributed on every axis but in different degrees. This is remarkable, because none of the measured characters could be used to determine sex, neither in larvae, nor in adults. In *Dysdercus fasciatus,* the 7th abdominal tergite length underwent sexual differentiation at the imaginal moult (Blackith *et al.,* 163, p. 320, table 1, variate 11).

The separation of the sexes on the first axis corresponds to the fact that overall size is, in the average, larger in females than in males, especially at the end of the development. Thus, sex first influences overall size.

The second canonical axis distinguishes between males and females at the end of the development, because of their different proportions for *E:* for an equal "size," *E* is relatively larger in males. Thus, sex also influences the relative proportions of hemelytra and leg segments.

Differences are evident between sexes along the third canonical variate. It has, however, to be kept in mind that this axis accounts for a small part of the total variance. The differences are nevertheless significant from the first stage where *Ta1/Ti2* ratio stands already in favour of the females.

Finally, on the fourth axis, male and female centroids separate at pre-imaginal and imaginal stages (V and A) because of the ratio *Ti3/Ta1 + Ti2* which is in favour of the females. This sex incidence on the 4th direction of variation occurs during the period of large increment of *E.* This seems to indicate a different response to juvenile hormone, according to sex, at the level of the proportions between the three leg segments. Since the hormone produces a general action on the metabolism (Slama, 1971), it is not surprising that its disappearance results also in an effect on the growth of non-imaginal characters (e.g., leg segments). Considering the growth rates of the leg segments, they are generally decreasing at the 4th and mostly at the imaginal moult (Cuzin-Roudy, 1975). This fall in growth rate does not affect the different segments equally and does not occur when larval growth is prolonged by an addition of a juvenile hormone analogue. Canonical discriminant analysis shows that the fall in growth rate affects,

differently according to sex, the proportions between some particular leg segments. The canonical analysis conducted on the 11 original variates (9 leg segments + R and E) confirms the last results. This shows that the stepwise procedure actually selected the most significant variates. In this analysis, male and female centroids are separated in the same way, at stage V and A, on the 4th axis, and variate *Ti3* lies on this axis in contrast to all the other variates (except for *F3* and *E*, whose structure coefficients are almost zero).

In conclusion, growth factors express themselves mostly on the first axis, which corresponds to the increment of all the dimensions. There is a joint effect of the sex factor on the first axis, more important at the end of the development. Dispersion on the second axis is mainly the reflection of the particular mode of growth of the hemelytra, under the direct effect of juvenile hormone, acting differently according to sex. Sexual disjunction is effective on the 3rd axis from stage I, on *Ta1/Ti2* proportions, a fact not obvious in bivariate analysis. Finally, an independent direction of variation (axis IV) can be attributed to a probably indirect effect of juvenile hormone on the leg segment growth rates, at the end of the development. It should be kept in mind that, if in some cases, one biological factor finds its main expression on a particular canonical axis, a strict correspondence between factors and canonical variates cannot generally be established. In fact, the different axes correspond to more or less independent responses of the variates to different biological factors having more or less prevalent effects along the four directions of variation.

Four canonical variates account for the most significant part of the data variation during growth in *Notonecta maculata*. Relying on bivariate allometry and on experimentation with juvenile hormone, they have been related with composite influence of biological factors such as general growth trend, sex differentiation and juvenile hormone. Canonical discriminant analysis constitutes a valuable tool for the study of the complex growth interactions between the measured characters, and yields a clear synthetic pattern of the influences and of the antagonisms that manifest themselves during the post-embryonic development of *Notonecta maculata*.

Acknowledgments

The authors wish to thank Dr. J. P. Scheidecker for the access to the computing facilities of the "Observatoire de Nice," and Drs. K.

Sláma and F. Sehnal (Entomological Institute of the Czechoslovak Academy of Sciences, Prague) who provided the juvenile hormone analogue. Dr. G. O. Mackie is acknowledged for his help with the English translation. The technical help of Mrs. M. Gegouzo and the editorial assistance of Mrs. C. Onteniente have been greatly appreciated.

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