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INDIVIDUAL VARIATION OF ONTOGENIES: A LONGITUDINAL STUDY OF GROWTH AND TIMING

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Abstract.—This study of growth and developmental time in the water strider *Limnoporus canaliculatus* (Heteroptera: Gerridae) is based on longitudinal data from specimens reared individually in the laboratory. I analyzed multivariate allometry using a common principal components approach. This technique identified patterns of variation that were uncorrelated both within and among instars and which remained fairly constant throughout the growth period; in contrast, the overall amount of variation increased from young to older instars. Negative correlations between size and subsequent growth increments indicated convergent growth in the first three instars, but there was a transition to positive correlations (divergent growth) in later instars. Analysis of covariation among measurements made in different instars showed strong ontogenetic autocorrelation and revealed patterns remarkably similar to those found in mammals and birds; yet corresponding analyses of growth increments showed mainly independent variation in different instars. Therefore, I conclude that the strong correlations among stage-specific measurements result from the part-whole relationships inherent to these cumulative size data, but do not reflect specific properties of the organisms studied. In contrast to size increments, instar durations of water striders were highly correlated throughout the larval period, indicating that individuals tended to develop at either relatively fast or relatively slow rates in all instars. The correlations between growth increments and instar durations were nil or negative, contrary to expectations from life-history theory. The results of these analyses of individual variation match the findings from other water striders and from interspecific comparisons in the genus *Limnoporus*, but information about physiological mechanisms of molting and growth in insects cannot completely explain the patterns observed.

Key words.—Age, allometry, common principal components, constraint, developmental time, Gerridae, growth, heterochrony, life history, morphometrics, ontogeny, size.

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The evolution of ontogeny has attracted much attention in recent years (Gould 1977; McKinney and McNamara 1991; Hall 1992). Interest in the evolution of organismic form motivated part of this research, because the diversity of morphological structures is the outcome of variation in growth and development. On the other hand, life-history studies include growth in size and the schedules of transitions between developmental stages as important elements of the relationships between organisms and their environment (Roff 1992; Stearns 1992).

Some studies in this field have compared the ontogenies of several taxa, viewing differences as the results of past evolutionary change (e.g., Creighton and Strauss 1986; Strauss 1990; Klingenberg and Spence 1993). Others have dealt with the patterns of variation of ontogenetic processes within populations that provide the potential for continuing microevolution (e.g., Cheverud et al. 1983; Lynch 1988; Kirkpatrick and Lofsvold 1989; Atchley and Hall 1991). In such analyses of ontogenetic variation, the lack of variability can be as important as its presence, because it constitutes a developmental constraint on the future evolution of the traits under consideration (Maynard Smith et al. 1985; Gould 1989; Kirkpatrick and Lofsvold 1992; Björklund 1993).

Developmental processes produce morphological variation and constraints and thus affect evolutionary processes in two principal ways. First, they determine the growth curves, that is, the functions relating age or developmental stage to morphological and physiological traits. As a consequence, they influence the extent to which values of the same trait at different ages can vary independently (Cheverud et al. 1983; Kirkpatrick and Lofsvold 1992; Björklund 1993). Second, because developmental processes often affect several traits simultaneously (Riska 1986), multivariate patterns of covariation among traits at a given age reflect these processes (e.g., Cheverud 1982a, 1995; Zelditch 1987; Cowley and Atchley 1990; Atchley et al. 1992; Paulsen and Nijhout 1993) and can in turn affect the potential for evolutionary change (Lande 1979; Cheverud 1984). These two aspects have been integrated in theoretical syntheses (Atchley and Hall 1991; Cowley and Atchley 1992; Atchley et al. 1994), but there are few detailed empirical studies that jointly consider covariation among traits and across developmental stages (but see Cheverud et al. 1983; Björklund 1993). Such studies were limited because statistical techniques specifically designed for longitudinal studies with multiple measurements have become available only recently (Klingenberg et al. 1996). Furthermore, the existing studies of ontogenetic variation and constraints have been based on analyses of age-specific size measurements, but few have examined the variation in growth increments (but see Riska et al. 1984).

Growth processes not only produce variation in morphometric traits, but they can also eliminate it by compensatory growth, such that all individuals converge toward a "target" size as adults (Tanner 1963), irrespective of differences in their earlier growth history. Differences in growth may be due to starvation (Wilson and Osbourn 1960; Blum et al. 1985) or individual variation apparent even under controlled laboratory conditions (Monteiro and Falconer 1966; Atchley 1984; Riska et al. 1984). Although vertebrates have been studied in the most detail, growth regulation has also been reported from insects (Tanaka 1981; Bryant and Simpson 1984), crustaceans (Hartnoll and Dalley 1981; West and Costlow 1987), and echinoderms (Ettensohn and Malinda 1993). Whereas some regulation of growth occurs through hormones

(Tanner 1963; Blum et al. 1985; Shea et al. 1990) and thus affects multiple measurements simultaneously, leading to tight overall integration, there is also ample evidence from a variety of studies that organs independently control their final size to a considerable degree (Bryant and Simpson 1984). Therefore, although growth regulation potentially is a major determinant of ontogenetic variation, it is not possible to predict what specific effects these processes have on the patterns of covariation among morphometric traits.

Ontogenetic variability is not limited to morphometric characters, but the timing of developmental events also can vary, which may lead to evolution by heterochrony (McKinney and McNamara 1991; Klingenberg unpubl.). To study this variation within and between populations, it is important to find appropriate standards for comparison. Yet studies of organisms with continuous growth most commonly are forced to ignore this variation, because they are usually based on measurements at fixed ages (e.g., Cheverud et al. 1983; Riska et al. 1984), even in comparisons between taxa (e.g., Creighton and Strauss 1986; Björklund 1993; Wayne and Ruff 1993). A possible alternative is standardization of age relative to an ontogenetic event, such as the peak velocity of growth in height for human adolescents (Cameron et al. 1994), but this may be very difficult if the event is gradual and if the data have high temporal resolution. To avoid such ambiguities, an ideal study system should have a fixed number of discrete developmental stages, a condition that is met by many hemimetabolous insects.

Water striders (Heteroptera: Gerridae) are excellent study organisms to address these questions because they can be reared individually in the laboratory, measurements of the cuticles cast during molting provide an accurate record of growth, and each molt is a distinct developmental event. Here I report the results of a longitudinal growth study in *Limnoporus canaliculatus* (Say). This study thus complements an earlier comparison of ontogenies among the six species of the genus *Limnoporus*, which revealed a considerable degree of interspecific variation in timing and extent of growth (Klingenberg and Spence 1993). I performed a joint analysis of covariation among measurements within and across developmental stages, using a new statistical model specifically designed for such studies (Flury and Neuenschwander 1995a; Klingenberg et al. 1996). Based on these results, I compare analyses of variation and constraint in growth increments to those in age-specific measurements, and I examine growth regulation and the relation between instar durations and growth in size.

Materials and Methods

This study is based on longitudinal data from all five larval instars (L1–L5) and adults of the water strider *L. canaliculatus*. Exuviae collected from bugs reared individually make it possible to obtain measurements from single individuals in all growth stages without manipulating the delicate larvae.

Laboratory Culture and Measurements

The water striders used in this study were the offspring of a sample of overwintered adults collected in Morris County, New Jersey, on May 1, 1992. The entire laboratory-rearing experiment was performed in the same climate-controlled room (20°C, photoperiod 16L:8D). Adults were kept as a mass culture, provided with Styrofoam strips for oviposition, and fed ad libitum with frozen flesh flies, *Neobelliera bullata* (Parker). Styrofoam strips were replaced regularly, and those with eggs in advanced stages of development were checked for hatched larvae at intervals of approximately 12 h.

Hatchlings were transferred into individual rearing containers (diameter 11.5 cm, height 8 cm), each with about 1 cm of water and a small piece of Styrofoam floating on the surface. Each larva was fed a frozen flesh fly daily; this is an ad libitum regime, as the weight of a fly far exceeds that of even an adult water strider. Larvae were checked for molts twice daily; the data for instar durations therefore have a resolution of approximately 12 h. After each molt, the cast exuvia was collected and subsequently stored in 70% ethanol. After the final molt, when the new cuticle had hardened, adults were killed by deep-freezing and later stored in 70% ethanol.

For this study, I analyzed measurements of the lengths of the femora and tibiae of the middle and hind legs. The cuticle of the legs is rigidly sclerotized; therefore, shrinking or other effects of preservation can be ruled out. Because antennal segments tended to telescope into one another in exuviae, their lengths, included previously in cross-sectional studies of growth in water striders (Klingenberg and Zimmermann 1992a; Klingenberg and Spence 1993), could not be measured reliably and were therefore not used in this study. Measurements were made with a video system attached to a dissecting microscope.

The data analyzed in this study are means of left and right body sides if both sides could be measured; if the value from one side was missing, the value measured on the other side was included. The combined variability from asymmetry and measuring error was small relative to the variation among individuals. The data set includes only those individuals for which all four variables could be measured on at least one body side in all five larval instars and the adult stage. I checked data for outliers and reexamined individuals with extreme values. Bugs with deformities, mostly because of abnormal molting, were excluded. Wing polymorphism did not have an influence on the results of this study. Most of the individually reared water striders were wingless: of the 89 females with complete data, only one was winged, and of the 70 males, five were winged (two of them brachypterous), although the mass culture in the same room produced a higher proportion of winged bugs. As preliminary univariate and multivariate analyses showed that winged individuals did not differ from the wingless ones either in morphometric traits or in instar durations, I included all bugs in this study regardless of wing morph.

All morphometric variables, but not the instar durations, were transformed to natural logarithms before the analyses.

Statistical Analyses

Longitudinal growth studies with multiple measurements are complex because there are correlations both within and across ontogenetic stages. If there are p measurements for each individual at k growth stages, the overall covariance matrix $\mathbf{S}$

has a pattern of k^2 blocks, each of dimension $p \times p$. The block $\mathbf{S}_{ij}(i,j=1,...,k)$, in the i-th row and the j-th column of $\mathbf{S}$, is a $p \times p$ matrix that contains the covariances of the p measurements in stage i with those in stage j ($\mathbf{S}'_{ij}=\mathbf{S}_{ji}$). The blocks along the diagonal, $\mathbf{S}_{ii}$, are the within-stage covariance matrices, as they have been used traditionally in both longitudinal and cross-sectional studies (e.g., Cuzin-Roudy 1975; Zelditch and Carmichael 1989; Klingenberg and Zimmermann 1992a). Conversely, analyses of the covariation among stages in one measurement at a time (e.g., Cheverud et al. 1983; Kirkpatrick and Lofsvold 1989,1992; Björklund 1993), say the h-th variable, consider only the h-th position along the diagonal in each of the blocks $\mathbf{S}_{ij}$.

Common Principal Component Model.—The basis of the statistical model I use here is the observation that patterns of variation among characters are often similar within several ontogenetic stages (e.g., Cuzin-Roudy 1975; Zelditch and Carmichael 1989; Klingenberg and Zimmermann 1992a). These studies considered only the blocks along the main diagonal of the composite covariance matrix, whereas the model used here extends this similarity to all blocks. The model assumes that the different stages share the same principal components, called common principal components (CPCs), and that different CPCs are uncorrelated not only within but also across developmental stages. For instance, the CPC1 in the L1 can be correlated only with the CPC1 in later instars but not with the CPC2 in any instar. Therefore, the transformation of the original variables to CPCs simultaneously renders diagonal all blocks of the patterned covariance matrix (see Klingenberg et al. 1996). This model, CPCs for dependent random vectors (Neuenschwander 1991; Flury and Neuenschwander 1995a), is an extension of the CPC model for independent groups (Flury 1988), which has been used in a number of morphometric studies (Airoldi and Flury 1988; Klingenberg and Zimmermann 1992a,b; Klingenberg and Spence 1993; Klingenberg 1996).

For longitudinal data, this CPC model can reduce a very complex analysis to a number of separate, simpler ones. As the CPCs are uncorrelated within and across stages, each block of the patterned covariance matrix of CPC scores is diagonal; the diagonal elements can be used to study covariation of CPCs among stages. Unlike the original measurements, in which separate analyses of the covariation across stages for each trait ignore the correlations between variables, each CPC can be analyzed in isolation without any loss of information. Like conventional principal component analysis (PCA), this CPC model can be useful as a tool for data reduction, because some components often account for only a minor fraction of the total variation and may be ignored. The fit of the model can be assessed either by tests based on asymptotic theory (Neuenschwander 1991) or by permutation tests (Klingenberg et al. 1996).

Klingenberg et al. (1996) have demonstrated the use of the CPC model for the data set of female *L. canaliculatus* and have discussed it in some detail; here I also apply it to the males, but I mention the statistical aspects only briefly. To estimate CPCs, I used a version of the orthogonal FG^+ algorithm (Flury and Neuenschwander 1995b) written in the SAS/IML language (this routine is available through the Internet: file://life.bio.sunysb.edu/morphmet/dcpc.exe.ibmpc). The CPCs were ordered by the average proportion of total variance within instars and covariance among instars for which they accounted (for details, see Klingenberg et al. 1996).

I tested the fit of the CPC model to the data with a permutation test. The key assumption of the model is that different CPCs are uncorrelated within and between instars, whereas corresponding CPCs can be correlated across instars. This can be used as the null hypothesis in a permutation test (Pitman 1937; Manly 1991; Good 1994). For each sex, I ran 1000 random permutations: the CPC scores were reshuffled separately for the CPC2–CPC4, but keeping all instars together, thus leaving unchanged the correlations among instars for each CPC. For each of the randomized data sets, I computed the CPCs and three different test statistics. The e statistic (Klingenberg et al. 1996) is a measure of overall deviation from the CPC model (for a detailed discussion, see Neuenschwander 1991; Flury and Neuenschwander 1995a,b). I used two additional statistics, the maximum absolute correlation and covariance between different CPCs, because they can pinpoint the CPCs and instars where deviations occur (note that ordinary significance tests do not apply here, because this is the highest absolute value of the 216 correlations or covariances between different CPCs). The null distributions of the test statistics from the permutation runs were then compared to the values from the original data.

To compute standard errors, I used the bootstrap method (Efron and Tibshirani 1993), with 250 iterations. A preliminary bootstrap analysis produced inflated standard errors because of changes in the ordering of CPCs and in the signs of their coefficients (see also Jackson 1993). To avoid this problem, I used a different rule to order the CPCs for the bootstrap routine and assigned each bootstrap CPC to that CPC of the original sample to which it was most similar, as judged by the magnitude of their inner product (this is equivalent to the use of angles between CPCs); these assignments were unambiguous in all 250 bootstrap runs for each sex. To prevent arbitrary changes in signs of CPC coefficients, the signs of all coefficients of the bootstrap CPC were reversed if the inner product of the original and corresponding bootstrap CPCs was negative.

Ontogenetic Allometry.—To take advantage of the information contained in the longitudinal data, I computed patterns of ontogenetic allometry in a manner slightly different from cross-sectional studies (e.g., Klingenberg and Zimmermann 1992a). Instead of a PCA of data pooled over individuals and stages, here I used a MANOVA of individuals and instars to separate static (individual) from ontogenetic variation. Therefore, a PCA of the between-instar matrix of sums of squares and cross-products can be used to analyze ontogenetic variation, and the resulting PC1 is a vector of ontogenetic allometry. To estimate standard errors, I used the bootstrap procedure, with 250 resampling iterations (random resampling among individuals, i.e., keeping the measurements from all instars together for each bug). A separate analysis was run for each sex.

Analyses of Variation and Constraints.—To study patterns of variation and identify possible constraints on the dynamics of growth in overall size, I used conventional PCAs of the covariance matrices of CPC1 scores in the six instars, of the

TABLE 1. Common principal-component coefficients and their bootstrap standard errors (in parentheses). Abbreviations of morphometric variables: MF, middle femur; MT, middle tibia; HF, hind femur; HT, hind tibia.

Variable	CPC1	CPC2	CPC3	CPC4
Females				
MF	0.471	0.436	−0.059	0.765
	(0.019)	(0.038)	(0.115)	(0.029)
MT	0.414	0.073	0.878	−0.229
	(0.024)	(0.049)	(0.062)	(0.136)
HF	0.491	0.465	−0.427	−0.600
	(0.026)	(0.042)	(0.108)	(0.084)
HT	0.605	−0.767	−0.209	0.048
	(0.030)	(0.031)	(0.052)	(0.054)
Males				
MF	0.491	0.462	0.003	0.738
	(0.031)	(0.038)	(0.082)	(0.022)
MT	0.388	0.059	0.870	−0.299
	(0.016)	(0.054)	(0.035)	(0.097)
HF	0.560	0.343	−0.475	−0.586
	(0.026)	(0.067)	(0.077)	(0.056)
HT	0.542	−0.816	−0.135	0.150
	(0.055)	(0.040)	(0.060)	(0.035)

increments in these "size" scores, and of instar durations. To estimate standard errors for these PCAs, I used the bootstrap with 250 resampling iterations. Inflated standard errors due to changes in the ordering of PCs and sign reversals of PC coefficients were a problem in some of these analyses, and as for the CPCs, I assigned the bootstrap PCs to the most similar PC in the original sample (i.e., the one with which it had the highest absolute inner product) and changed signs if the inner product was negative. Similar analyses were conducted for the CPC2 scores and increments.

For analyzing growth and its regulation, I derived a log-size variable (Mosimann 1970) from the CPC1 by rescaling its coefficients such that they summed up to unity; its antilogarithm therefore scaled as a linear dimension (Klingenberg and Zimmermann 1992b; Klingenberg and Spence 1993). I computed growth ratios as the antilogarithm of the difference in the log-size scores between successive instars. More intuitively, this measure of growth can be interpreted as the postmolt/premolt ratio for overall size. The geometric mean of these ratios in a sample is the geometric-mean growth ratio, which can also be obtained from cross-sectional studies (Klingenberg and Zimmermann 1992b; Klingenberg and Spence 1993). As a measure of relative size within instars, I used the ratio of the individual's size to the geometric mean size in that instar, computed as the antilogarithm of the difference of each individual's log-size score from the instar mean score.

Correlations among relative size, postmolt/premolt ratios, and instar durations are Pearson product-moment correlations. I tested them against the null hypothesis of independence with two-tailed permutation tests (Pitman 1937), each with 10,000 random permutations (see also Manly 1991; Good 1994). I present correlations with their original *P*-values, but to determine statistical significance, I use the sequential Bonferroni adjustment (Rice 1989) to control for experimentwise error rate within each sex (tablewide $\alpha = 0.05$).

TABLE 2. Percentages of total variance for which the CPCs account within each instar, and their bootstrap standard errors (in parentheses).

Instar	CPC1	CPC2	CPC3	CPC4
Females				
L1	75.9	13.8	6.7	3.6
	(3.8)	(2.6)	(1.5)	(0.8)
L2	79.7	11.9	5.3	3.1
	(3.8)	(2.6)	(1.2)	(0.7)
L3	76.8	14.0	5.4	3.9
	(3.9)	(2.7)	(1.3)	(0.9)
L4	79.7	13.2	5.1	1.9
	(3.5)	(2.6)	(1.1)	(0.5)
L5	79.8	13.2	5.2	1.8
	(3.3)	(2.5)	(1.2)	(0.5)
Ad.	78.2	14.4	5.8	1.5
	(3.6)	(2.8)	(1.4)	(0.4)
Males				
L1	76.3	16.2	4.0	3.6
	(3.6)	(3.1)	(0.7)	(0.9)
L2	79.0	13.2	5.0	2.8
	(2.6)	(2.3)	(1.1)	(0.5)
L3	81.2	10.5	4.8	3.5
	(3.0)	(2.2)	(1.3)	(0.7)
L4	80.5	11.7	5.2	2.6
	(3.6)	(2.4)	(1.4)	(0.6)
L5	81.9	12.0	4.5	1.6
	(4.4)	(4.1)	(1.4)	(0.3)
Ad.	79.8	13.7	4.6	1.9
	(3.6)	(3.0)	(1.3)	(0.6)

RESULTS

Static Variation: Common Principal Components

The analyses in both sexes produced similar results (Table 1). The CPC1 is a size axis, whose coefficients are all positive and of similar magnitude. The CPC2 shows a contrast of the middle and hind femora against the hind tibia. The CPC3 opposes the middle tibia to the hind femur and, to a lesser extent, to the hind tibia. Finally, the CPC4 contrasts the middle femur to the middle tibia and, more strongly, to the hind femur. The close congruence of results between sexes is even more apparent from vector correlations between pairs of CPCs, which all exceed 0.99; the corresponding angles between CPC axes range from 5.7° to 7.8°. The CPC estimates are fairly stable, as indicated by their standard errors. The standard errors obtained with the bootstrap method are fairly similar (differences < 0.03) to those from a jackknife analysis (for females only; Klingenberg et al. 1996).

All four CPCs account for fairly constant proportions of variation throughout ontogeny (Table 2). The CPC1 takes up more than 75% of the total variation in all instars and both sexes. A moderate amount of variation, about 10–16%, is associated with the CPC2, whereas the other two CPCs show substantially less variability. These values are close to the proportions of total variance for which the PCs accounted in separate PCAs in each instar and sex (for the females, this comparison is presented in Klingenberg et al. 1996).

The permutation tests reveal some deviations from the CPC model for both sexes. In females, all three test statistics are significant, but the deviations concern exclusively the CPC3, which accounts only for a small proportion of variance, and

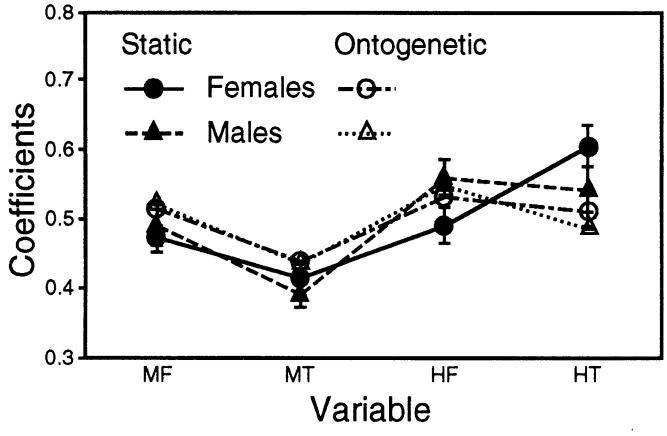

FIG. 1. Comparison of static and ontogenetic allometry. Joint patterns of static allometry in all six instars were estimated by the CPC1 coefficients, and ontogenetic allometry by the PC1s of the between-instar matrices from MANOVA. Error bars indicate bootstrapped standard errors (250 iterations); note that those for the ontogenetic PC1s are extremely small (all ≤ 0.001) and entirely covered by the symbols.

will not be considered here (Table 2; for details, see Klingenberg et al. 1996). In males, the e statistic indicates a significant deviation from the CPC model overall ($e = 405.9$; $P < 0.001$), and the maximum absolute covariance between different CPCs (value 8.48; $P = 0.01$) attributes it to the relatively large covariance between CPC1 and CPC2 in adults, which both have large variances (none of the other combinations of CPCs have similarly large covariances). Nevertheless, the maximum absolute correlation does not indicate a statistically significant deviation from independence (maximal $|r| = 0.36$, CPC1 in L1 with CPC3 in L2; $P = 0.19$). In both sexes the first two CPCs, which account for about 90% of the variation in each instar, can be considered uncorrelated within and between instars, despite the misfit of the model overall, and therefore can be studied separately in the subsequent analyses.

Relations between Static and Ontogenetic Allometry

In analyses of ontogenetic allometry, the PC1s account for the overwhelming majority of variation, 99.7% in females and 99.8% in males, and thus indicate that the model of simple allometry fits the data very well. The patterns of ontogenetic and static allometry are similar (Fig. 1), as indicated by angles of 4.5° and 6.5° between ontogenetic PC1 and static CPC1 in males and females, respectively (vector correlations > 0.99). The angles between the ontogenetic PC1 and the static PC1s for each instar are narrowest in the L2 or L3 instars, and increase toward the adult stage.

Individual Variation in Growth

To assess the patterns of instar-specific variability in overall size, I performed a PCA of the CPC1 scores in each instar. The first PC alone accounts for about three-quarters of the total size variation in both sexes (Fig. 2). This PC1 is an axis summarizing variation in general growth performance: all instars have positive coefficients (Fig. 2), indicating that individuals tend to be either relatively large or relatively small

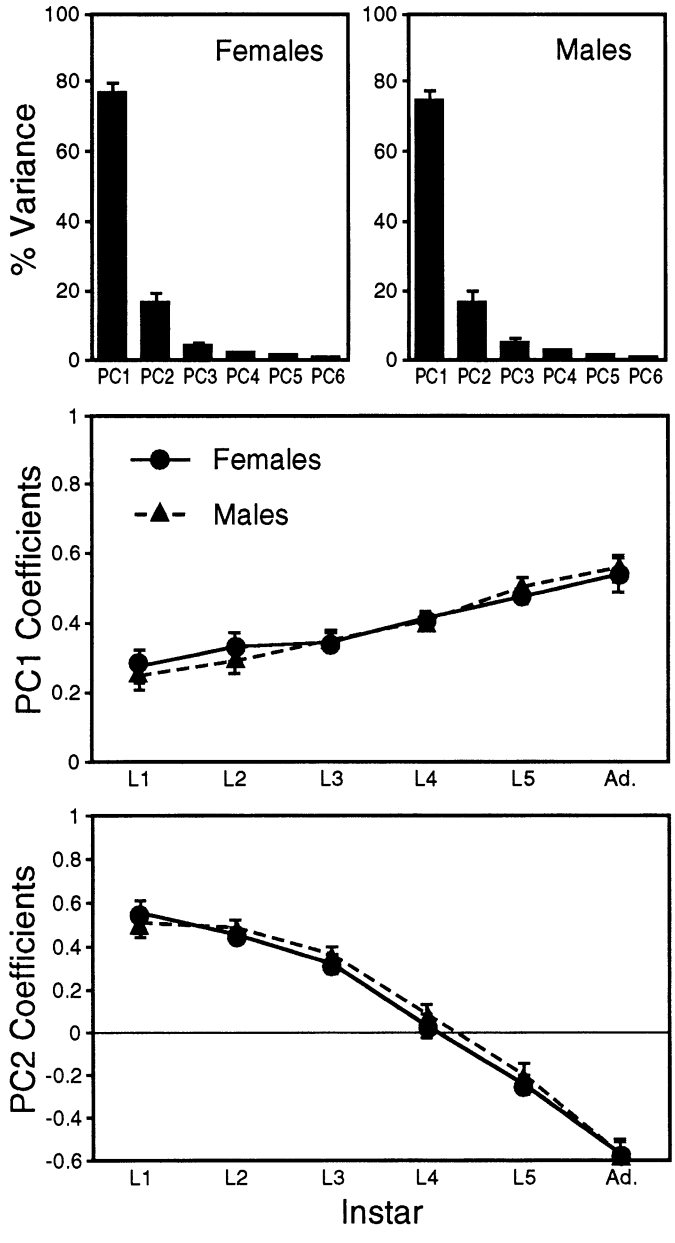

FIG 2. Variation and constraint in instar-specific size. The PCA used the covariance matrix of individual CPC1 scores in all six instars. The top panels show the eigenvalues, expressed as percentages of total variance; note that the PC1 takes up most of the variation. The middle and bottom panels show the coefficients of each instar on the PC1 and PC2, respectively. The PC1 has positive coefficients for sizes in all instars and shows variation in overall growth, whereas PC2 contrasts size in early versus late instars. Error bars indicate the bootstrapped standard errors of the estimates; some of the standard errors are so small that the error bars are too short to be seen or the symbols entirely cover them.

in all instars. The PC1 coefficients gradually increase from instar to instar, manifesting variation in slope that is associated with the height of growth curves, that is, larger bugs also tend to have the steeper growth curves. The PC2, which takes up about 17% of the total variance in both sexes, is the only other PC accounting for a relatively large proportion of variation. It contrasts the overall size scores in early against

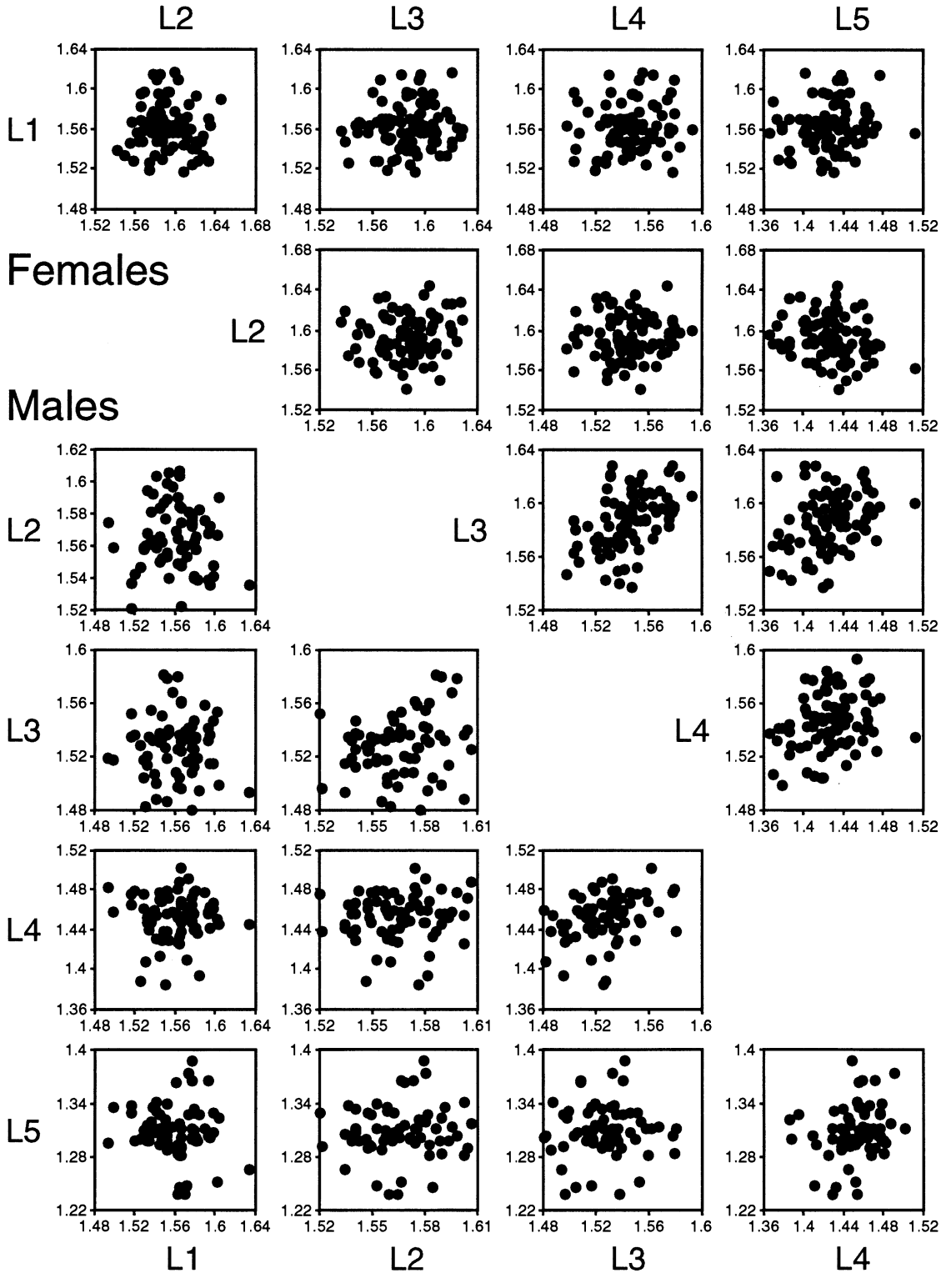

FIG. 3. Relations between growth increments of individual water striders in different instars. Plotted values are the postmolt/premolt ratios of a multivariate size measure that scales as a linear dimension (see text for details).

those in late instars in a graded series (Fig. 2), showing that growth curves vary in slope, but not in shape, as the profile of PC2 coefficients is fairly straight. The PC2 thus features variation only in the slope of growth curves; it can be visualized as a movement where the entire growth curve oscillates like a "seesaw" pivoting about the fairly constant size in the L4 instar, but does not "bend." The remaining PCs account only for small proportions of variance.

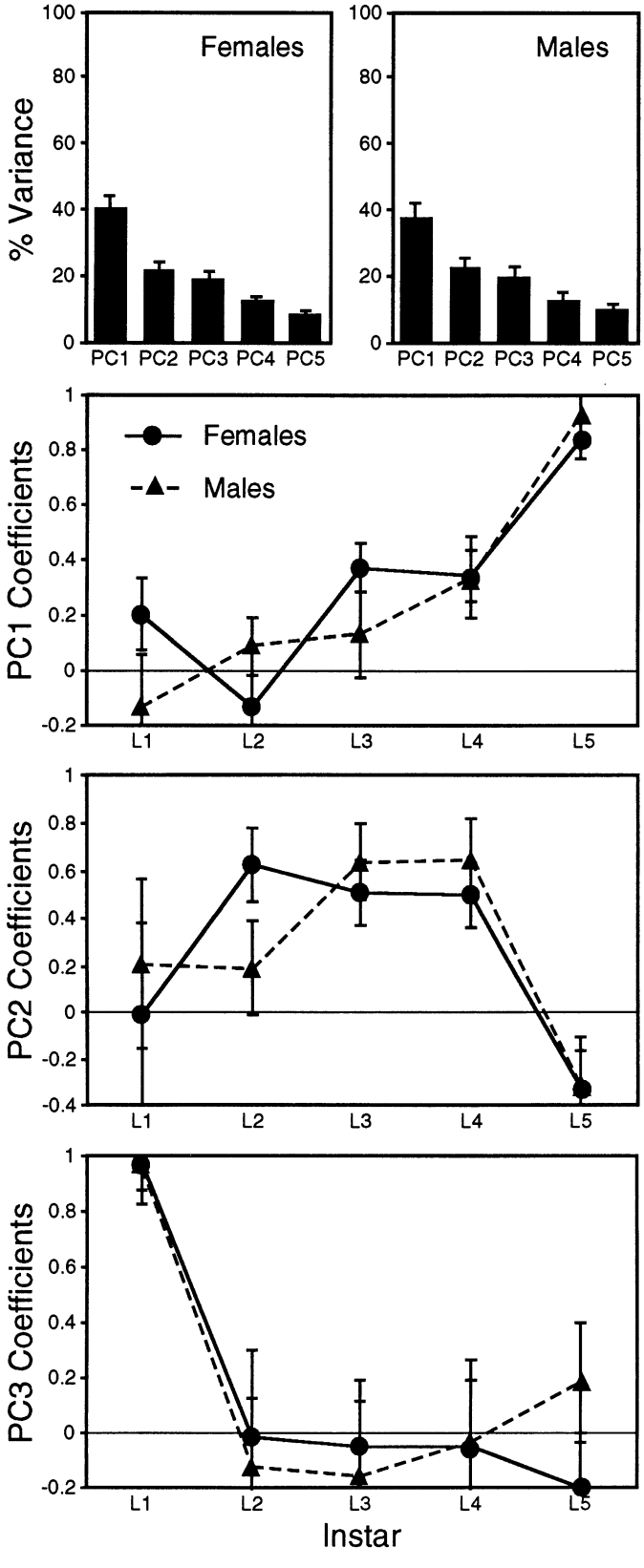

FIG. 4. Patterns of variation in size increments. The PCA was based on the covariance matrix of differences in CPC1 scores between successive instars. The eigenvalues, shown as percentages of the total variation (top panels), indicate that the PC1 is less dominant in this analysis of increments than in the corresponding analysis for instar-specific CPC1 scores (Fig. 2). The lower three panels show the coefficients of each instar on the PC1, PC2, and PC3; none of these is an axis featuring variation in growth performance throughout ontogeny. Error bars are bootstrapped standard errors of the respective estimates.

Given the dominance of the PC1 in the preceding analysis, it is rather surprising that the growth increments are not strongly correlated between instars (Fig. 3). In females, correlations between postmolt/premolt ratios of the overall size variable range from −0.22 (L2–L5) to 0.47 (L3–L4; P = 0.0001); the latter and the correlation between L4 and L5 increments (r = 0.32; P = 0.002) are the only ones significantly different from zero after sequential Bonferroni adjustment for the 10 pairwise correlations. In males, correlations range from −0.09 (L1–L5) to 0.34 (L3–L4; P = 0.004, and thus significant after adjustment; all others nonsignificant).

The PCAs of increments in CPC1 scores reflect this weak covariation, as the PC1s of increments account for much smaller fractions of the total variation (Fig. 4) than in the analyses of instar-specific size (Fig. 2). Moreover, the larger standard errors and the incomplete congruence of results between the sexes indicate that patterns are less well-defined. In the analysis of increments, none of the PCs is an axis of variation in growth performance in all instars jointly (Fig. 4). Instead, the PC1s almost exclusively feature the variability in late growth, as they have much larger coefficients for the L5 than for earlier instars (Fig. 4). The PC2s emphasize increments in the L3 and L4 (and L2 in females), and contrast this to L5 growth, whereas the size increase in the first molt alone dominates the PC3s. Although the remaining two PCs account for smaller portions of variation, both are associated with appreciable variability. Therefore, this PCA provides no evidence of any substantial constraints on growth increments, in contrast to the analyses of instar-specific size, where variation is largely concentrated in just two dimensions.

Postmolt/premolt ratios also are at most moderately correlated with relative size in the previous instar (Fig. 5). In the L1 through L3 instars, correlations between relative size and growth ratios are negative, and thus indicate compensatory growth (females: L1 r = −0.25, P = 0.015, marginally nonsignificant after sequential Bonferroni adjustment; L2 r = −0.28, P = 0.007; males: L1 r = −0.33, P = 0.004; the latter two correlations remain significant after adjustment for five tests in each sex). Yet, because these correlations are rather weak, compensatory growth is just strong enough to maintain the variance of the CPC1 approximately constant in the L1 and L2 instars (in females also the L3). In the L3, compensatory growth eliminates less variation than the new growth increments generate, and therefore, morphometric variance increases from the L3 to the L4 instar. In the final two molts, the correlations between size in the previous instar and growth are positive, although not statistically significant, and the variance of the CPC1s increases by 36–51% in each molt.

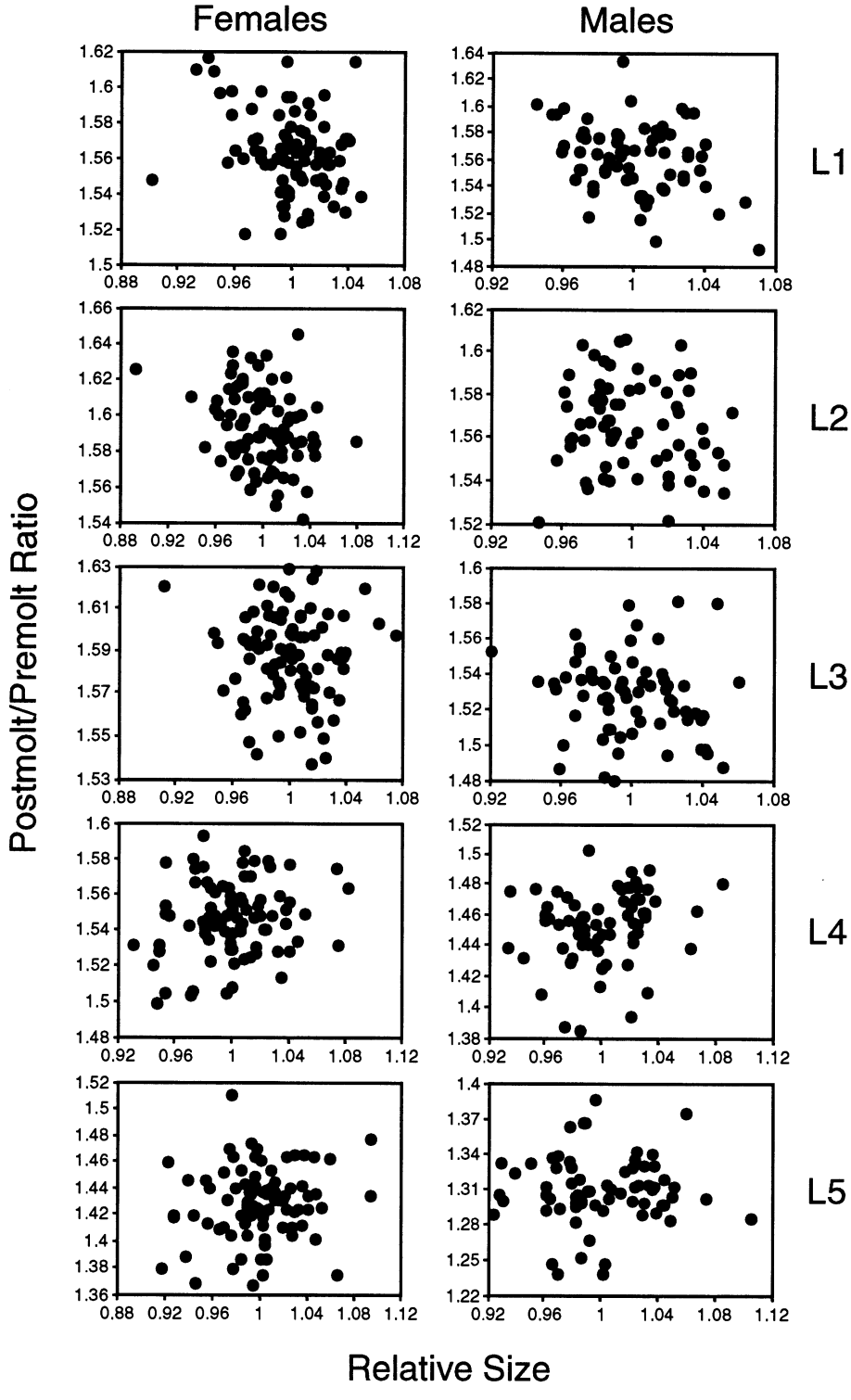

FIG. 5. Relations between relative size in an instar and the size increment in the following molt. Relative size is the ratio between an individual's score for the multivariate size variable and the geometric mean for this variable in that instar and sex, and the postmolt/premolt ratio is the ratio between the size scores in this and the following instar. There are some negative correlations of size and growth ratio in early instars, indicating compensatory growth, but not in later instars.

To examine whether the patterns of ontogenetic variation in size-independent variables are the same as for the "overall size" component, I performed PCAs of the scores and increments of the CPC2. The results are similar to the corresponding analyses for the CPC1. For the CPC2 scores, the PC1s account for 61% and 58%, and the PC2s for 22% and 21% of the total variance in females and males, respectively. The PC1 coefficients are all positive and increase in mag-

nitude from early to late instars and thus indicate that bugs tend to have either high or low CPC2 scores in all instars, whereas the PC2 coefficients gradually decline from positive to negative values, indicating "seesaw-like" variation of CPC2 growth trajectories. In the analyses of CPC2 increments, like in those for the CPC1, the PC1s account for less of the total variation (39% and 53% in females and males, respectively), and each PC mainly features the increment during one instar, contrasting it with CPC2 changes in other instars. Correlations between CPC2 scores in one instar and the differences to the next instar show that there clearly is growth regulation for this size-free component of variation (with correlations as strong as $r = -0.55$). In females, these correlations are significantly negative in the L1, L2, and the L3, whereas in males the L1, L2, and L5 instars have significant negative correlations (all after sequential Bonferroni correction).

Instar Durations

Unlike the size increments, instar durations are clearly correlated among instars, and the range of instar durations tends to increase from younger to older stages (Fig. 6). Correlations range from 0.47 (L1–L4) to 0.85 (L2–L4) in females and from 0.38 (L1–L2) to 0.88 (L3–L4) in males. All these correlations are highly significant, and remain so even after Bonferroni correction for the 10 tests, as none of the permutation runs matches the observed values ($P < 0.0001$), except for three correlations in males (P-values between 0.0001 and 0.0015, all significant after sequential Bonferroni adjustment). Altogether, these correlations show that developmental rates of individuals vary consistently in all instars, in contrast to the results found for the size increments (above).

In accordance with these correlations, the PC1 of the covariance matrix of instar durations takes up almost all the total variance (Fig. 7). All instars have positive PC1 coefficients, which gradually increase from the L1 to the L5, reflecting the larger variation in later instars. The results for both sexes are very similar, and the small standard errors indicate that they are statistically stable.

A PCA of cumulative developmental time, that is, the ages at the five molts, shows an even stronger dominance of the PC1 (Fig. 8). As in the preceding analysis, the PC1 coefficients increase from instar to instar, but this increase is steadier and somewhat stronger here.

Instar durations are only weakly correlated with the size increments in the same instar (Fig. 9). Correlation coefficients are negative or very close to zero, and only those in the L5 instars (and L3 in males) retain statistical significance after sequential Bonferroni adjustment (females, L5, $r = -0.34$, $P = 0.0006$; males, L3, $r = -0.39$, $P = 0.0006$; L5, $r = -0.32$, $P = 0.006$). Yet these correlations are not significant for growth from hatching to the final molt, as relative size of adults and total developmental time are uncorrelated (females $r = -0.14$, $P = 0.18$; males $r = -0.14$, $P = 0.24$).

Discussion

This study integrates approaches that traditionally have been used separately to study variation in ontogeny. Longitudinal growth data for multiple traits provide the basis for jointly analyzing allometry, morphological integration, growth regulation, and the variation in growth trajectories. In this discussion, I attempt to synthesize the results of these analyses, linking them to the knowledge on growth processes in hemimetabolous insects and to the evolutionary patterns found in a comparison of ontogenies among all six species of the genus *Limnoporus* (Klingenberg and Spence 1993).

Patterns of Covariation among Morphometric Variables

The analysis with CPCs demonstrates that patterns of static variation are fairly constant throughout postembryonic development. Moreover, these patterns also account for similar proportions of the total variation, and it is mainly the overall amount of variability that increases from early to late instars. The CPC1 accounts for more than three-quarters of the total variance within each stage, showing that the model of simple allometry fits well in all instars, and that morphological integration is fairly tight. Similarity of static allometry in several instars also has been found for other water striders (Klingenberg and Zimmermann 1992a) and for a backswimmer (Cuzin-Roudy 1975). Besides constant components of static covariance structure, however, other organisms also show substantial changes associated with key ontogenetic events (e.g., Zelditch 1988; Zelditch and Carmichael 1989).

The patterns of static and ontogenetic allometry are very similar (Fig. 1). There are two possible explanations for such similarities if both length and dircetion of growth vectors between successive stages vary (Teissier 1948; Cock 1966; Cheverud 1982b; Klingenberg and Zimmermann 1992a). First, if growth increments along the average allometric trajectory, but not in perpendicular directions, are positively correlated with size in the preceding instar, then static and ontogenetic allometry will be more similar in the following instar, and will eventually become identical (Cock 1966). Because the static and ontogenetic patterns tend to be more similar in younger instars (especially the L2) than in later ones, this explanation does not correspond well to the observed patterns. Second, static and ontogenetic allometry will be similar if variation in the direction of growth vectors is uncorrelated with variation in length (Cheverud 1982b). Regression analyses of the lengths and direction coefficients of growth vectors are significant only for the L3 in females and the L4 in males (after Bonferroni corrections), and R^2 values generally are low. Correlations between direction and length of growth vectors may result from the slight curvature of growth trajectories (Klingenberg and Zimmermann 1992a; Klingenberg and Spence 1993). Although this indicates that there are specific sources of variation during each growth stage, the close overall congruence of static and ontogenetic allometry suggests that variability in the extent of growth contributes most to static variation.

Phenotypic Variation and Constraints in Growth Curves

There are two ways to analyze data measured in each of a number of developmental stages: by stage-specific values and by the increments between successive stages (see also Riska et al. 1984; Lynch 1988; Cowley and Atchley 1992). Although stage-specific values are simply the sum of the value in the first stage and later increments as they accumulate

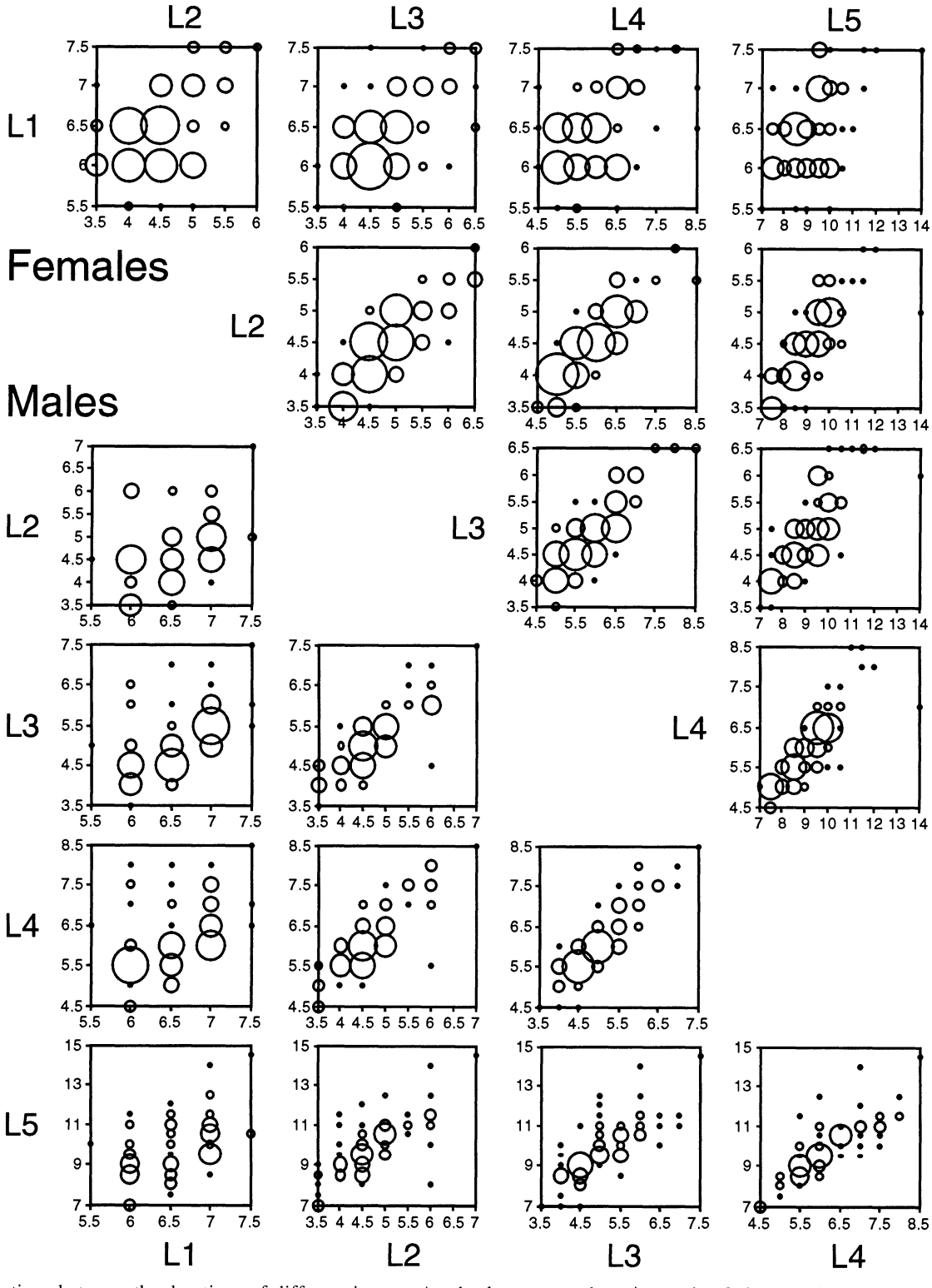

FIG. 6. Relations between the durations of different instars. As checks were made at intervals of about 12 h, the temporal resolution of the data is relatively coarse, and there is extensive overlap of data points. In this graph, therefore, the diameter of the "bubbles" is proportional to the number of individuals with a particular combination of instar durations. Note the increasing variation in instar duration and the successively stronger correlations between instars.

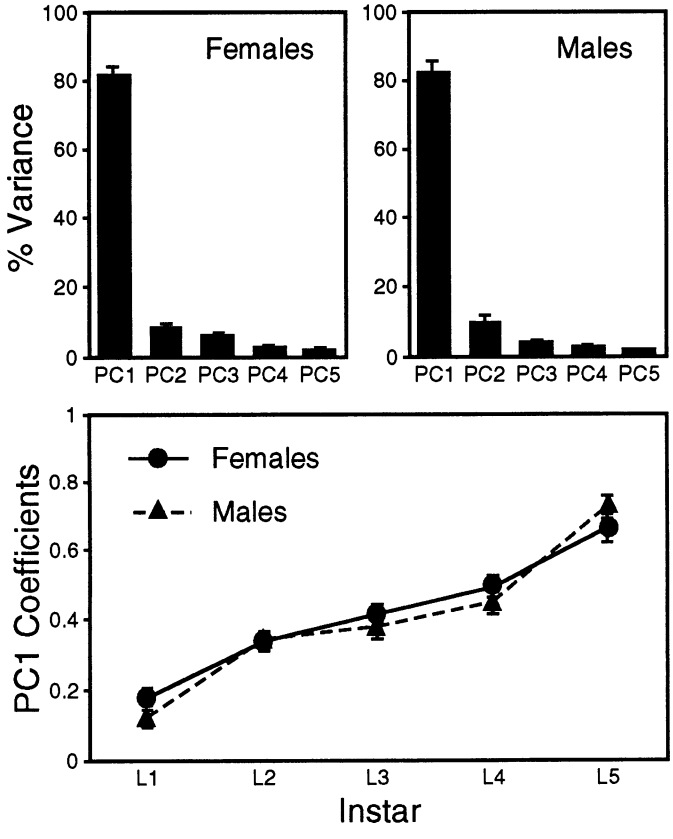

FIG. 7. Patterns of variation in instar durations. The PCA used the covariance matrix of untransformed instar durations. The top panels show the eigenvalues, expressed as percentages of total variance; note the strong dominance of the PC1. The bottom panel shows the coefficients of each instar on the PC1. Error bars indicate the bootstrapped standard errors of the estimates; for a few of the PC1 coefficient values, the symbols entirely cover the error bars.

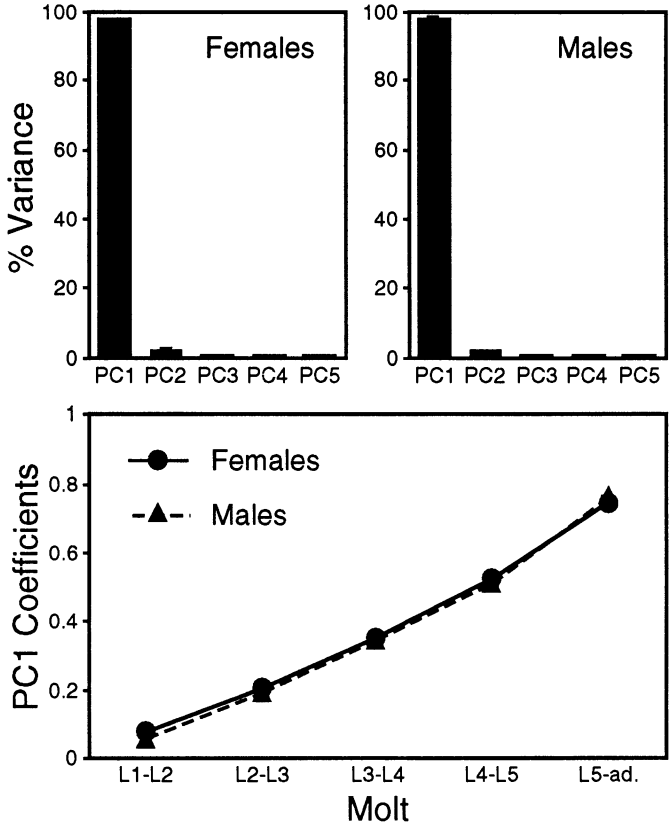

FIG. 8. Patterns of variation in cumulative developmental time. The data used in this PCA were the ages at which the five molts of each individual took place; the corresponding incremental values are the instar durations, except for the first variable (age at L1–L2 molt), which is identical for both sets (it is also the L1 duration). The top panels show the eigenvalues, expressed as percentages of total variance; note the dominance of the PC1 is even stronger than in the analysis shown in Figure 6. The bottom panel shows the coefficients of each instar on the PC1. Standard errors are so small that the symbols entirely cover the error bars.

through the growth period, I have shown by direct comparison that analyses of such cumulative and incremental data can produce very different results.

In the analysis of cumulative size, the PC1 strongly dominates (Fig. 2), suggesting a well-integrated ontogeny and constrained phenotypic variation of growth trajectories. The bulk of the variation affects growth rates in all instars jointly, producing variability in the overall height and in the slopes of growth curves. Only a much smaller fraction (the PC2) is "seesaw" variation contrasting the size in early versus late instars, which gives the growth curves a certain degree of variation in slopes independent of height. The remaining four PCs account for negligible amounts of variation. Conversely, analyses of growth increments suggest there is very little ontogenetic integration but that increments in different instars are largely independent of each other (Figs. 3, 4). In this analysis, not a single PC is an axis of overall growth performance; instead, the first three PCs separately feature variability of growth in the late, middle, and early larval period, respectively (Fig. 4). Moreover, the PCs differ much less in the amount of variance they account for, and there clearly is variation in all five dimensions. Unlike the cumulative size data, this analysis of increments provides very little evidence for phenotypic constraints in ontogenies. The cumulative and incremental analyses for the CPC2 produced similar differences; this finding therefore applies not only to the "size" variable.

The stark contrast between these two kinds of analyses demonstrates the difference between cumulative and incremental data. In cumulative data, there is a strong ontogenetic autocorrelation, because the value of a variable in a particular stage is the sum of the value at the preceding stage and the intervening growth increments (see Riska et al. 1984). For instance, if X denotes size in a given instar and Y the growth increment, the covariance between the sizes in this and the following instars is

$$\mathrm{cov}(X, X + Y) = \mathrm{var}(X) + \mathrm{cov}(X, Y).$$

This formula shows that the covariance between successive instars is bound to be high unless there is a large negative covariance between X and Y, which would imply tight regulation of size (see below).

In a number of aspects, my findings for cumulative size in water striders remarkably resemble those reported in studies of genetic and phenotypic covariance or correlation ma-

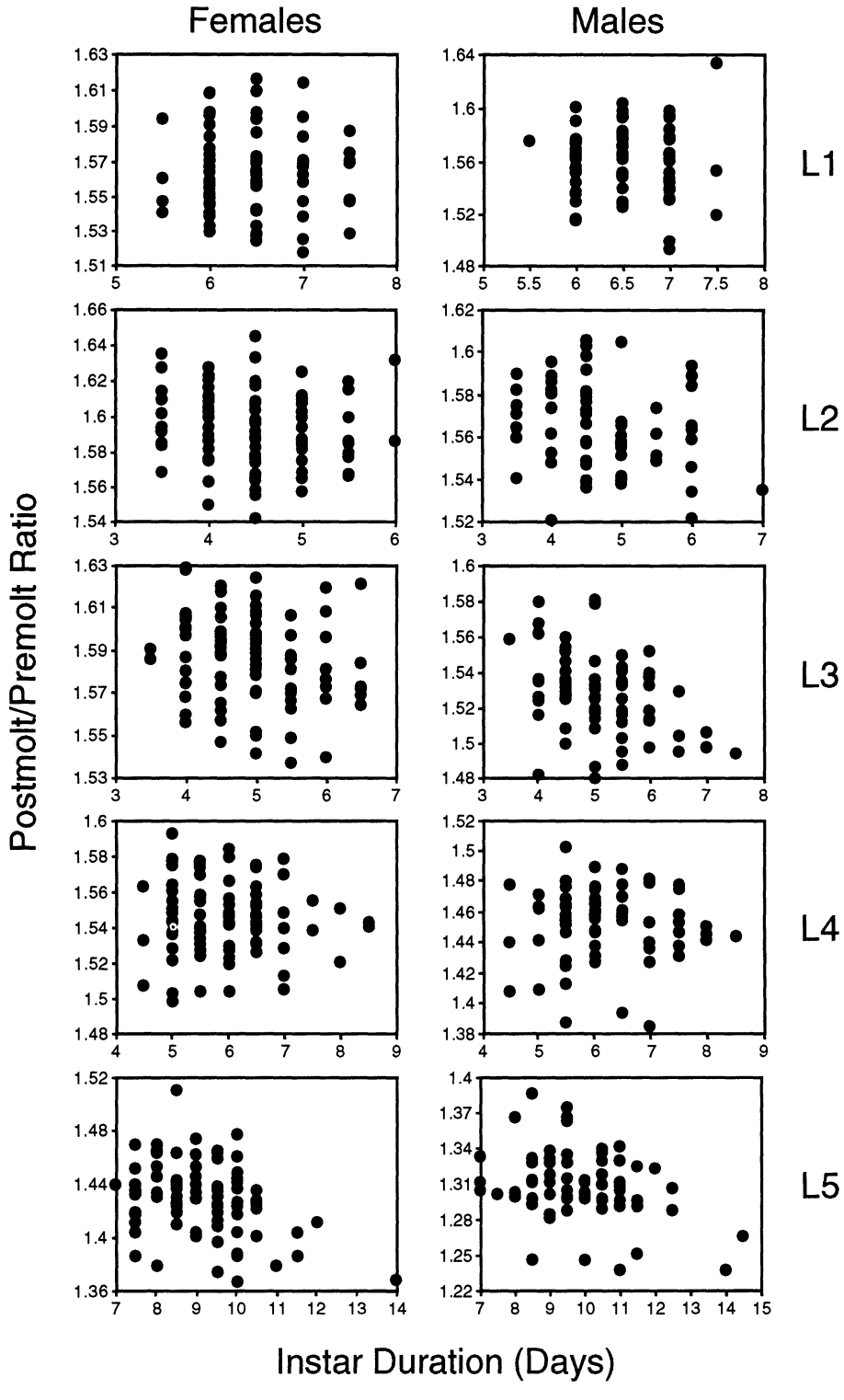

FIG. 9. Relations between instar durations and size increments in the following molt. Postmolt/premolt ratios are for the multivariate size variable. The correlations are either very close to zero or negative (especially in the L3 and L5 instars).

trices for mammals and birds (Cheverud et al. 1983; Leamy and Cheverud 1984; Kirkpatrick and Lofsvold 1989, 1992; Björklund 1993; although the latter three studies used an infinite-dimensional approach, I refer to the eigenfunctions as "PCs" in this comparison). In all of these studies, the PC1 alone accounts for almost all the total variance, and the PC1 coefficients are positive for all age groups, indicating joint variation in size at all ages. Furthermore, in several analyses the PC2s have coefficients that steadily increase or decrease with age in a graded series, with opposite signs at

the youngest and oldest ages (see Fig. 2; Cheverud et al. 1983; Björklund 1993; additional examples have the extreme coefficient values at the second-youngest or second-oldest age, and are thus very close to a graded series, see Kirkpatrick and Lofsvold [1992]). This shows that "seesaw" variation in growth trajectories is not limited to insects.

These similarities for analyses of cumulative data, which range across taxa with such drastically different modes of growth as water striders, rodents, and finches, raise the question whether analyses of incremental data for those other examples would produce as little evidence for ontogenetic integration as for water striders. Published phenotypic and genetic correlation matrices for weight in mice (tables 3, 4 in Riska et al. 1984) provide an opportunity to compare PCA results for cumulative and incremental data directly. For both sexes, and for phenotypic as well as genetic correlations, the PC1 of cumulative data accounts for about three-quarters of the total variance or more, and its coefficients indicate variation in height of the growth trajectory; the PC2s, which take up 9–20% of the total variance, display a "seesaw" pattern of variation. The corresponding analyses of incremental data, however, suggest less severe constraints: the PC1s take up about 25% of the variation in phenotypic, and 50–60% in genetic correlation matrices. The PC coefficients mostly feature contrasts among increments in particular stages; none of them shows easily interpretable patterns like those of the cumulative weights. This congruence between different data sets suggests that the constraints identified in these studies are not a consequence of the underlying physiological or genetic mechanisms, but of the autocorrelation among measurements in successive growth stages.

The fairly tight integration seen in cumulative analyses is straightforward, given the nature of these data. Variation in growth increments at any stage appears again in all subsequent stages, unless subsequent growth compensates for it. Growth variability acting only in the earliest stages therefore contributes to the variation in height of the growth trajectory, in addition to processes affecting growth throughout ontogeny. Likewise, increases or decreases of growth rates mainly at intermediate ontogenetic stages will contribute to "seesaw" patterns. Ontogenetic autocorrelation tends to spread the effects of an ontogenetic event at a particular stage to the successive stages and thus causes integration and constraints of ontogenetic variation.

Clearly, these constraints on variation are real, even if caused by such autocorrelation, and they can influence the evolution of ontogeny (Cheverud et al. 1983; Kirkpatrick and Lofsvold 1992; Björklund 1993). Because ontogenetic autocorrelation is due to the part-whole relationship inherent in cumulative data and applies to every growth trajectory analyzed in this way, unless there is perfect compensatory growth, the resulting constraints are universal (in the sense of Maynard Smith et al. 1985).

Nevertheless, my study shows that there can be substantial variation in growth increments even in cases in which such constraints exist, but this variability may not be apparent in cumulative data as it is swamped with morphometric variation accumulated from earlier stages. The differences in growth trajectories among water striders of the genus *Limnoporus* show that variation was sufficient for size increments in successive instars to evolve relative to each other even in opposite directions (Klingenberg and Spence 1993). This is particularly obvious between *L. canaliculatus* and its sister species *Limnoporus esakii* (Andersen and Spence 1992), which grows much more between the L3 and L4, but less between the L4 and L5 instars (fig. 7 in Klingenberg and Spence 1993).

Regulation of Growth

A variety of animals have the ability to regulate growth to achieve a particular target size, including mammals (e.g., Tanner 1963; Riska et al. 1984; Cameron et al. 1994) and arthropods (e.g., Hartnoll and Dalley 1981; Tanaka 1981; Bryant and Simpson 1984; West and Costlow 1987; Lynch 1988; Freeman 1990). By compensating for variability in earlier stages, whether of genetic or environmental origin, such regulatory growth reduces the variance around the "target" value. The covariance between a measurement in one stage (X) and the subsequent growth increment (Y) plays an important role for determining the variance of the trait in the following stage, because

$$\mathrm{var}(X + Y) = \mathrm{var}(X) + 2\mathrm{cov}(X, Y) + \mathrm{var}(Y).$$

Because var(X) and var(Y) are always positive, a negative cov(X, Y) is the only factor that can keep the morphometric variance constant or even reduce it from one stage to the next (for further discussion, see Riska et al. 1984). The same covariance also relates size regulation to the issue of ontogenetic autocorrelation (see above).

Body size, as indicated by the CPC1 scores, is negatively correlated with subsequent growth in the youngest instars (Fig. 5), indicating convergent growth (Riska et al. 1984). In females, the covariance between the CPC1 scores in the L1 and the following growth increment is just sufficient to maintain about the same variance of CPC1 scores in the L2 [i.e., $2\mathrm{cov}(X, Y) \approx -\mathrm{var}(Y)$]. In the L2, this covariance is even negative enough to produce a slight decrease in the variance of CPC1 scores from the L2 to the L3 instar. In males, there is a similar decrease in variance between the L1 and L2. The remaining correlations between size and increments up to the L3 instar are also negative, but not significantly different from zero; the corresponding covariances are not sufficiently negative to compensate for the new variance of CPC1 scores produced by variable growth. There is divergent growth (Riska et al. 1984) in the L4 and L5, and the correlations between CPC1 scores and increments are positive, although not significantly different from zero. Despite the relatively low correlations, ranging from 0.04 to 0.21, the corresponding covariances contribute 13–45% of the increase in variance during these instars.

Similar changes occur in several arthropods for which growth regulation has been shown. Several studies have found convergent growth in some stages of development, but not in others, for the German cockroach (Tanaka 1981), several crustaceans (Hartnoll and Dalley 1981; West and Costlow 1987; Lynch 1988; Freeman 1990), and rodents (Atchley 1984; Riska et al. 1984). Changes in growth regulation therefore are a widespread phenomenon; however, the underlying

changes in mechanisms of growth control and the possible adaptive significance are poorly undestood.

Variability in Timing of Molts

The variation in instar durations is substantial, especially in later instars. The slowest-developing bugs can spend up to twice as much time in an instar than the fastest ones (Fig. 6). Unlike size increments, instar durations are clearly correlated, and show strong integration (Fig. 7). Therefore, most variation is confined to the overall rate of development, indicating that bugs tend to have either relatively short or relatively long intervals between molts in all instars. This result is especially remarkable given the fact that instar durations are incremental traits; in the corresponding analysis of cumulative ages at the five molts (days after hatching), the phenotypic constraint is even more pervasive due to serial autocorrelation (Fig. 8).

This large intraspecific variability corresponds to the interspecific variation in the genus *Limnoporus*, which suggests that instar durations have been evolutionarily plastic (Klingenberg and Spence 1993). The species I have studied here, *L. canaliculatus*, is the fastest developing of the six species; in most instars, the slow-growing *Limnoporus genitalis* has instar durations that are about twice as long (Klingenberg and Spence 1993). Geographic variation within species (Fairbairn 1984; Firko 1986; Blanckenhorn 1991; Blanckenhorn and Fairbairn 1995) and interspecific variation (Spence et al. 1980) further underscore the evolutionary flexibility of development time among water striders.

The correlations between instar durations and growth increments were nil to moderately negative (Fig. 9), and the correlations between total developmental time and adult size were negative as well, but not significantly different from zero. None of these correlations was positive, as many theoretical models of life-history evolution assume, and as they have been found especially in comparisons across higher taxa (Stearns 1992; Roff 1992). Although my result contradicts these model assumptions, it is consistent with observations in other water striders species (Blanckenhorn and Fairbairn 1995; Klingenberg and Spence 1996) and with the lack of a clear relationship between size and development time among *Limnoporus* species (Klingenberg and Spence 1993).

Heterochronic changes of ontogeny can affect the rates or timing of growth (Alberch et al. 1979; McKinney and McNamara 1991; Klingenberg unpubl.). The possibility to distinguish individual variation in timing of developmental events, such as the molts, from variation in size at a given stage makes hemimetabolous insects excellent systems for studying the evolution of ontogenies. The data from water striders suggest that these parameters vary independently, both within populations (this study) and among species (Klingenberg and Spence 1993). To understand the mechanisms of heterochronic change fully, however, both the genetic covariances and patterns of natural selection would have to be known.

Studies of trait-at-age data have implicitly assumed that animals at a given age are in comparable developmental stages. My results demonstrate that this assumption does not hold generally, but needs to be examined case by case (see also Hall and Miyake 1995). In some instances, the resolution of longitudinal analyses can be improved by adjusting the time axis to a developmental event (e.g., Cameron et al. 1994), but even the sequence of developmental events can change among related species (e.g., Strauss 1990), making it difficult to identify homologous stages (see also Alberch 1985). Studies that explicitly consider the timing of multiple developmental events in combination with age-specific size will help to better understand the evolution of ontogeny.

Possible Physiological Mechanisms

Data from many organisms demonstrate size regulation by convergent growth. In water striders, regulation occurs by accelerating or slowing growth rates, not by altering instar durations, as there is no positive correlation between instar durations and growth increments (Fig. 9). The CPC1 and the CPC2 scores both showed targeted growth, but not always in the same instars. Thus, regulation seems to affect the overall size of the organism and the relative sizes of its parts in separate ways, suggesting that the corresponding mechanisms act locally. Yet the nature of these mechanisms remains unclear.

Molting in Heteroptera is induced by stretching of the abdominal wall (Nijhout 1979, 1994), and larger larvae must therefore attain a larger size than smaller ones to trigger a molt. Moreover, the size of the old cuticle and the degree to which it is stretched in the early phase of a molting cycle influence the size in successive instars, because the old cuticle functions as a template when the new one is laid down (Bennet-Clark 1971). Both stretch-induced molting and the template effect are more likely to produce divergent growth rather than growth regulation by convergent growth.

The mechanism of stretch-induced molting may partly explain the negative correlations between developmental time and growth increments (Klingenberg and Spence 1996). Bugs with higher growth rates reach the size threshold sooner than slower-growing ones and therefore initiate the new molting cycle earlier. Because the period from initiation of the molting cycle to the formation of the new cuticle is fairly constant and independent of feeding (Blakley and Goodner 1978), growth that takes place during this period influences the size of the following instar. Consequently, bugs with higher growth rates also increase more in size after initiation of the molting cycle than those with slower growth. Together, the earlier molt and larger growth increment of faster-growing individuals lead to a negative correlation between instar durations and growth increments, and to divergent growth.

My data from water striders are only partially consistent with these expected patterns. Clearly, this mechanism does not apply in the youngest instars when there is convergent growth; thus, it is not surprising that significant negative correlations between instar durations and size increments do not occur before the L3 instar. It is perplexing, however, why the correlations in the L4 instar are so weak, although there are stronger negative correlations in the L3 and L5 instars in both sexes. Moreover, the correlations between total developmental time and adult size are weak as well.

Overall, these mechanisms of insect growth may account for a part, but clearly not for all the patterns of ontogenetic

variation in this data set. This reflects the poor current understanding of the processes controlling postembryonic growth. Although the physiology of growth in Heteroptera is relatively well known, because true bugs have been used as model organisms in this field (mainly late larval instars of *Rhodnius* and *Oncopeltus*), much remains unknown. Clearly, the topics of growth regulation and the correlation between growth in size and developmental time are a promising field for study in both physiology and evolutionary biology.

Conclusions

In this study I simultaneously have considered the covariation of morphometric variables within and among instars, using a model of CPCs specifically suited for longitudinal data (Klingenberg et al. 1996). Analyses of cumulative and incremental data differ dramatically, demonstrating the pervasive influence of serial autocorrelation between successive instars. The patterns of phenotypic variation I found for cumulative growth curves of water striders are similar to those described from studies of both phenotypic and genetic constraints in mammals and birds (Cheverud et al. 1983; Kirkpatrick and Lofsvold 1992; Björklund 1993). Moreover, the analysis of published data for mice (Riska et al. 1984) revealed similar discrepancies between incremental and cumulative data. I argue that the patterns common to all these cumulative data sets are universal constraints due to the inherent part-whole relations; they may severely limit the variation of growth trajectories, but inferences about developmental processes based on such data should be made with caution. In contrast, the analyses of growth increments demonstrate substantial freedom for independent variation in all instars, which corresponds well to the variation observed among *Limnoporus* species (Klingenberg and Spence 1993).

The large variability in instar durations shows that the timing of developmental events differs extensively among individuals. Therefore, age and the instars provide two very different frames of reference for analyzing growth; intrinsic and extrinsic time are clearly different (Hall and Miyake 1995; Klingenberg unpubl.). Individual variability in *L. canaliculatus* corresponds to the interspecific variation found across this genus, in which several heterochronic changes have been found (Klingenberg and Spence 1993). Unlike size increments, instar durations are correlated among instars, indicating that individual variation in developmental rates is consistent throughout the larval period.

Overall growth in size switches from a convergent or targeted mode to divergent growth, and as a consequence, the variance in size remains constant during the first three instars, but increases sharply in later stages. There is a weak negative correlation between instar durations and size increments in some instars, which may be due to stretch-induced molting. Nevertheless, the physiological mechanisms and the genetic basis of ontogenetic variation remain mostly unclear. Much remains to be done before there can be an integrated understanding of patterns and processes in the evolution of ontogeny; this study of growth and developmental time is a step toward that goal.

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Literature Cited

Airoldi, J.-P., and B. K. Flury. 1988. An application of common principal component analysis to cranial morphometry of *Microtus californicus* and *M. ochrogaster* (Mammalia, Rodentia). J. Zool. (Lond.) 216:21–36.

Alberch, P. 1985. Problems with the interpretation of developmental sequences. Syst. Zool. 34:46–58.

Alberch, P., S. J. Gould, G. F. Oster, and D. B. Wake. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317.

Andersen, N. M., and J. R. Spence. 1992. Classification and phylogeny of the Holarctic water strider genus *Limnoporus* Stål (Hemiptera, Gerridae). Can. J. Zool. 70:753–785.

Atchley, W. R. 1984. Ontogeny, timing of development, and genetic variance-covariance structure. Am. Nat. 123:519–540.

Atchley, W. R., and B. K. Hall. 1991. A model for development and evolution of complex morphological structures. Biol. Rev. Camb. Philos. Soc. 66:101–157.

Atchley, W. R., D. E. Cowley, C. Vogl, and T. McLellan. 1992. Evolutionary divergence, shape change, and genetic correlation structure in the rodent mandible. Syst. Biol. 41:196–221.

Atchley, W. R., S. Xu, and C. Vogl. 1994. Developmental quantitative genetic models of evolutionary change. Dev. Genet. 15: 92–103.

Bennet-Clark, H. C. 1971. The cuticle as a template for growth in *Rhodnius prolixus*. J. Insect Physiol. 17:2421–2434.

Björklund, M. 1993. Phenotypic variation of growth trajectories in finches. Evolution 47:1506–1514.

Blakley, N., and S. R. Goodner. 1978. Size-dependent timing of metamorphosis in milkweed bugs (*Oncopeltus*) and its life history implications. Biol. Bull. 155:499–510.

Blanckenhorn, W. U. 1991. Life-history differences in adjacent water strider populations: Phenotypic plasticity or heritable responses to stream temperature? Evolution 45:1520–1525.

Blanckenhorn, W. U., and D. J. Fairbairn. 1995. Life history adaptation along a latitudinal cline in the water strider *Aquarius remigis* (Heteroptera: Gerridae). J. Evol. Biol. 8:21–41.

Blum, J. W., W. Schnyder, P. L. Kunz, A. K. Blom, H. Bickel, and A. Schurch. 1985. Reduced and compensatory growth: Endocrine and metabolic changes during food restriction and refeeding in steers. J. Nutr. 115:417–424.

Bryant, P. J., and P. Simpson. 1984. Intrinsic and extrinsic control of growth in developing organs. Q. Rev. Biol. 59:387–415.

Cameron, N., P. Gordon-Larsen, and E. M. Wrchota. 1994. Longitudinal analysis of adolescent growth in height, fatness, and fat patterning in rural South African black children. Am. J. Phys. Anthropol. 93:307–321.

Cheverud, J. M. 1982a. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516.

———. 1982b. Relationships among ontogenetic, static, and evolutionary allometry. Am. J. Phys. Anthropol. 59:139–149.

———. 1984. Quantitative genetics and developmental constraints on evolution by selection. J. Theor. Biol. 110:155–171.
———. 1995. Morphological integration in the saddle-backed tamarin (*Saguinus fuscicollis*) cranium. Am. Nat. 145:63–89.
Cheverud, J. M., J. J. Rutledge, and W. R. Atchley. 1983. Quantitative genetics of development: Genetic correlations among age-specific trait values and the evolution of ontogeny. Evolution 37:895–905.
Cock, A. G. 1966. Genetical aspects of metrical growth and form in animals. Q. Rev. Biol. 41:131–190.
Cowley, D. E., and W. R. Atchley. 1990. Development and quantitative genetics of correlation structure among body parts of *Drosophila melanogaster*. Am. Nat. 135:242–268.
———. 1992. Quantitative genetic models for development, epigenetic selection, and phenotypic evolution. Evolution 46:495–518.
Creighton, G. K., and R. E. Strauss. 1986. Comparative patterns of growth and development in cricetine rodents and the evolution of ontogeny. Evolution 40:94–106.
Cuzin-Roudy, J. 1975. Étude de la variabilité et de l'allométrie de taille chez *Notonecta maculata* Fabricius (insectes, hétéroptères), par les méthodes classiques et par la méthode des composantes principales. Arch. Zool. Exp. Gén. 116:173–227.
Efron, B., and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman and Hall, New York.
Ettensohn, C. A., and K. M. Malinda. 1993. Size regulation and morphogenesis: A cellular analysis of skeletogenesis in the sea urchin embryo. Development 119:155–167.
Fairbairn, D. J. 1984. Microgeographic variation in body size and development time in the water strider, *Limnoporus notabilis*. Oecologia 61:126–133.
Firko, M. J. 1986. Comparative life history evolution in the waterstrider, *Gerris remigis*. Ph.D. diss. Univ. of Pennsylvania, Philadelphia.
Flury, B. 1988. Common principal components and related multivariate models. Wiley, New York.
Flury, B. D., and B. E. Neuenschwander. 1995a. Principal component models for patterned covariance matrices, with applications to canonical correlation analysis of several sets of variables. Pp. 90–112 *in* W. J. Krzanowski, ed. Recent advances in descriptive multivariate analysis. Oxford Univ. Press, Oxford.
———. 1995b. Simultaneous diagonalization algorithms with applications in multivariate statistics. Pp. 179–206 *in* R. Zahar, ed. Approximation and computation: A festschrift in honor of Walter Gautschi. Birkhäuser, Boston, MA.
Freeman, J. A. 1990. Molt increment, molt cycle duration, and tissue growth in *Palaemonetes pugio* Holthuis larvae. J. Exp. Mar. Biol. Ecol. 143:47–61.
Good, P. 1994. Permutation tests: A practical guide to resampling methods for testing hypotheses. Springer-Verlag, New York.
Gould, S. J. 1977. Ontogeny and phylogeny. Harvard Univ. Press, Cambridge, MA.
———. 1989. A developmental constraint in *Cerion*, with comments on the definition and interpretation of constraint in evolution. Evolution 43:516–539.
Hall, B. K. 1992. Evolutionary developmental biology. Chapman and Hall, London.
Hall, B. K., and T. Miyake. 1995. How do embryos measure time? Pp. 3–20 *in* K. J. McNamara, ed. Evolutionary change and heterochrony. Wiley, Chichester, UK.
Hartnoll, R. G., and R. Dalley. 1981. The control of size variation within instars of a crustacean. J. Exp. Mar. Biol. Ecol. 53: 235–239.
Jackson, D. A. 1993. Stopping rules in principal components analysis: A comparison of heuristical and statistical approaches. Ecology 74:2204–2214.
Kirkpatrick, M., and D. Lofsvold. 1989. The evolution of growth trajectories and other complex quantitative characters. Genome 31:778–783.
———. 1992. Measuring selection and constraint in the evolution of growth. Evolution 46:954–971.
Klingenberg, C. P. 1996. Multivariate allometry. Pp. 23–49 *in* L. F. Marcus, M. Corti, G. J. P. Naylor, A. Loy, and D. E. Slice, eds. Advances in morphometrics. Plenum Press, New York.
Klingenberg, C. P., and J. R. Spence. 1993. Heterochrony and allometry: Lessons from the water strider genus *Limnoporus*. Evolution 47:1834–1853.
———. 1996. On the role of body size for life history evolution. Ecol. Entomol. In press.
Klingenberg, C. P., and M. Zimmermann. 1992a. Static, ontogenetic, and evolutionary allometry: A multivariate comparison in nine species of water striders. Am. Nat. 140:601–620.
———. 1992b. Dyar's rule and multivariate allometric growth in nine species of waterstriders (Heteroptera: Gerridae). J. Zool. (Lond.) 227:453–464.
Klingenberg, C. P., B. E. Neuenschwander, and B. D. Flury. 1996. Ontogeny and individual variation: Analysis of patterned covariance matrices with common principal components. Syst. Biol. 45:135–150.
Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33:402–416.
Leamy, L., and J. M. Cheverud. 1984. Quantitative genetics and the evolution of ontogeny. II. Genetic and environmental correlaions among age-specific characters in randombred house mice. Growth 48:339–353.
Lynch, M. 1988. Path analysis of ontogenetic data. Pp. 29–46 *in* B. Ebenman and L. Persson, eds. Size-structured populations. Springer-Verlag, Berlin.
Manly, B. F. J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, London.
Maynard Smith, J., R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert. 1985. Developmental constraints and evolution. Q. Rev. Biol. 60:265–287.
McKinney, M. L., and K. J. McNamara. 1991. Heterochrony: The evolution of ontogeny. Plenum Press, New York.
Monteiro, L. S., and D. S. Falconer. 1966. Compensatory growth and sexual maturity in mice. Animal Production 8:179–192.
Mosimann, J. E. 1970. Size allometry: Size and shape variables with characterizations of the lognormal and generalized gamma distributions. J. Am. Stat. Assoc. 65:930–945.
Neuenschwander, B. 1991. Common principal components for dependent random vectors. Ph.D. diss. Univ. of Bern, Bern, Switzerland.
Nijhout, H. F. 1979. Stretch-induced moulting in *Oncopeltus fasciatus*. J. Insect Physiol. 25:277–281.
———. 1994. Insect hormones. Princeton Univ. Press, Princeton, NJ.
Paulsen, S. M., and H. F. Nijhout. 1993. Phenotypic correlation structure among elements of the color pattern in *Precis coenia* (Lepidoptera: Nymphalidae). Evolution 47:593–618.
Pitman, E. J. G. 1937. Significance tests which may be applied to samples from any populations. II. The correlation coefficient test. J. R. Stat. Soc. B 4:225–232.
Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.
Riska, B. 1986. Some models for development, growth, and morphometric correlation. Evolution 40:1303–1311.
Riska, B., W. R. Atchley, and J. J. Rutledge. 1984. A genetic analysis of targeted growth in mice. Genetics 107:79–101.
Roff, D. A. 1992. The evolution of life histories: Theory and analysis. Chapman and Hall, New York.
Shea, B. T., R. E. Hammer, R. L. Brinster, and M. R. Ravosa. 1990. Relative growth of the skull and postcranium in giant transgenic mice. Genet. Res. (Camb.) 56:21–34.
Spence, J. R., D. H. Spence, and G. G. E. Scudder. 1980. The effects of temperature on growth and development of water strider species (Heteroptera: Gerridae) of central British Columbia and implications for species packing. Can. J. Zool. 58:1813–1820.
Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, Oxford.

STRAUSS, R. E. 1990. Heterochronic variation in the developmental timing of cranial ossifications in poecilid fishes (Cyprinodontiformes). Evolution 44:1558–1567.

TANAKA, A. 1981. Regulation of body size during larval development in the German cockroach, *Blatella germanica*. J. Insect Physiol. 27:587–592.

TANNER, J. M. 1963. Regulation of growth in size in mammals. Nature 199:845–850.

TEISSIER, G. 1948. La relation d'allométrie: Sa signification statistique et biologique. Biometrics 4:14–48.

WAYNE, R. K., AND C. B. RUFF. 1993. Domestication and bone growth. Pp. 105–131 *in* B. K. Hall, ed. Bone. Vol. 7: Bone growth—B. CRC Press, Boca Raton, FL.

WEST, T. L., AND J. D. COSTLOW. 1987. Size regulation of the crustacean *Balanus eburneus* (Cirripedia: Thoracica). Mar. Biol. 96:47–58.

WILSON, P. N., AND D. F. OSBOURN. 1960. Compensatory growth after undernutrition in mammals and birds. Biol. Rev. Camb. Philos. Soc. 35:324–363.

ZELDITCH, M. L. 1987. Evaluating models of developmental integration in the laboratory rat using confirmatory factor analysis. Syst. Zool. 36:368–380.

———. 1988. Ontogenetic variation in patterns of phenotypic integration in the laboratory rat. Evolution 42:28–41.

ZELDITCH, M. L., AND A. C. CARMICHAEL. 1989. Ontogenetic variation in patterns of developmental and functional integration in skulls of *Sigmodon fulviventer*. Evolution 43:814–824.

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