Biological Journal of the Linnean Society, 2002, **77**, 523–533. With 4 figures

Quantification of sexual dimorphism in *Asellus aquaticus* (Crustacea: Isopoda) using outline approaches

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Received 11 March 2002; accepted for publication 13 August 2002

A marked sexual dimorphism is often observed in arthropods species in which males perform precopulatory mate guarding. It is generally thought to reflect the influence of sexual selection. Until now, sexual dimorphisms associated with mate guarding have mainly been qualitatively described. However, assessing the effects of sexual selection on sexual dimorphims requires a preliminary quantitative assessment of differences in morphology between sexes. Using Fourier analyses, we tested if morphological dimorphisms could be quantitatively assessed in the isopod *Asellus aquaticus*. In addition, we checked whether sexual dimorphism in shape was exclusively related to mate guarding through considering characters that are not, *a priori*, implicated in mating behaviour. To assess the potential role of sexual selection in shaping morphology, we then examined how dimorphic characters could influence males' pairing success. Three characters (pleotelson, paraeopods 4 and 5) differed significantly in shape between males and females. In addition, two characters (pleotelson and paraeopods 4) differed in shape between guarding males and non-guarding males, with the latter being closer in shape to females. This suggests that sexual selection may be partly responsible for the observed morphological divergence between sexes in *A. aquaticus*. © 2002 The Linnean Society of London, *Biological Society of the Linnean Society*, 2002, **77**, 523–533.

ADDITIONAL KEYWORDS: allometry – complex series – Fourier – geometric morphometrics – head – mate guarding – multivariate analyses – paraeopods – pleotelson – sex – sexual selection – shape – size.

INTRODUCTION

Sexual dimorphism is a widespread phenomenon that affects morphological, physiological and behavioural traits (Philip & Foster, 1971; Andersson, 1994; Walker & Rypstra, 2001). Many theoretical and empirical studies have focused on the adaptive significance of these sexual dimorphisms (Gould, 1974; Slatkin, 1984; Hedrick & Temeles, 1989; Katsikaros & Shine, 1997; Abraham, 1998; Green, 2000; Walker & Fell, 2001). While some secondary sexual characters are quite obvious, such as the exaggerated tail of peacocks, others are more subtle and quantitative analyses are then required to identify them and to appraise the selective forces responsible for their evolution. This is often the case when the divergences between males and females concern the shape of characters, given that such differences between sexes can be very subtle and difficult to describe. Since morphological dimorphic structures are common, attention has been paid to such traits in a wide range of organisms (David, Laurin & De Ridder, 1988; Abell *et al.*, 1999; Forslund, 2000; Wellborn, 2000; Bonnet *et al.*, 2001) but the magnitude of the dimorphisms has mainly been examined using conventional metrical approaches that consist of linear distances. Such traditional morphometric methods provide an accurate description of the structure of interest when it is regular (such as circles, rectangles, etc.), but do not account for the overall form (Lestrel, 1997).

When shape is more complex, which is a frequent situation in biology, some crucial information could be lost. To improve on traditional techniques, new morphometric methods have been developed over the last 20 years (Kuhl & Giardina, 1982; Lestrel, 1989;

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Bookstein, 1991; Rohlf & Marcus, 1993; Marcus & Corti, 1996; Lestrel, 1997). These methods consider the whole geometry of the studied structures and provide an accurate measure of the shape of complex morphological forms. Two main approaches are available in relation to the nature of the descriptors: landmark approaches and outline approaches. Landmarks are specific points of the biological structure that are supposed to be equivalent or homologous between each specimen. Thus, shape variation among individuals could be assessed from the differences in the geometry of landmark configurations. With outline approaches, the shape is reduced to the outline of the structure that is defined by a set of constructed points located on the boundary. Outlines are then fitted by mathematical functions and the parameters of these functions can be employed to compare individual shapes between themselves. Geometric morphometric methods have proved to be powerful tools for comparing shapes at different taxonomic levels, including the intraspecific level (e.g. Lestrel, Bodt & Swindler, 1993; Loy, 1996; Arnqvist, 1998; Crônier *et al.*, 1998; Hard *et al.*, 2000; Alibert *et al.*, 2001; Monti, Baylac & Lalanne-Cassou, 2001), suggesting that such methods could be particularly useful for detecting and quantifying hidden morphological sexual dimorphisms.

In the present study, we investigated shape dimorphism in the freshwater crustacean *Asellus aquaticus* Linné (Isopoda). In this species, copulation is preceded by a period, called mate guarding, during which a male guards a female by carrying her until insemination becomes possible. Mate guarding is a competitive strategy used by males when a female's fertilization is time limited (Grafen & Ridley, 1983; Yamamura, 1987). Morphological dimorphisms associated with this mating behaviour are well documented in crustaceans, particularly in Gammaridae (Conlan, 1991). Such dimorphisms have also been reported in *A. aquaticus*. For example, males first pair of paraeopods, which is used to grasp their mate, bears apophyses that are absent in females. Similarly, the fourth pair of paraeopods of males, which allows them to carry females during mate guarding, is reduced and curved (Vandel, 1926; Balesdent, 1964). However, as for many other species of animals, these morphological dimorphisms have only been qualitatively described, which limits the possibility of assessing how variation in these traits influences individual fitness. The first aim of the present paper was to investigate if reported morphological dimorphisms could be quantitatively demonstrated. For that purpose, geometric methods were required since the first and fourth pairs of paraeopods are, obviously, characters for which traditional morphometric approaches would lead to an incomplete description of shapes. Since few landmarks were available in the structures of interest, the outline approach was preferred. In addition, we intended to check whether sexual dimorphism in shape was exclusively related to *A. aquaticus* mating behaviour. In this species, reported morphological dimorphisms concern exclusively characters that are used by males during the mate guarding. Therefore, we also examined other characters that are not, *a priori*, implicated in mate guarding, thus allowing comparison of shapes of these characters between guarding and non-guarding males.

MATERIAL AND METHODS

SAMPLING AND DATA ACQUISITION

Sampling was performed in the Ouche river at Echenon (Echenon: 47°19′N, 05°03′E; Burgundy, Eastern France) in April 2000. *Asellus aquaticus* were collected with hand nets using the kick sampling method (Hynes, 1954) and immediately preserved in an solution containing 70% ethanol and 5% glycerol for later measuring. Each precopula pair (i.e. a guarding male and the guarded female) was isolated in distinct tubes.

We measured the body size of all captured animals. This measure (estimated as a linear measurement from the front of the head to the tip of the pleotelson, Fig. 1) was taken by gently flattening the specimens between two microscope slides (Steel, 1961) and using an optic measuroscope (Nikon measuroscope 10×, Nippon KOGAKU K.K. MODEL 0.1/100 mm).

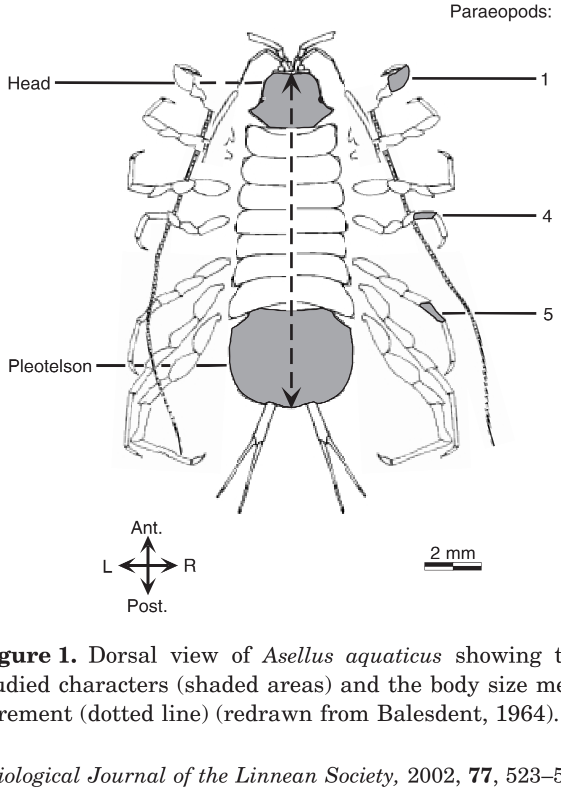

Figure 1. Dorsal view of *Asellus aquaticus* showing the studied characters (shaded areas) and the body size measurement (dotted line) (redrawn from Balesdent, 1964).

Sexual dimorphism in shape was appraised on the basis of five character measurements (head, pleotelson, propodos of the paraeopods 1, carpos of the paraeopods 4 and 5, Fig. 1). For bilateral characters (i.e. paraeopods), shape was only studied on the right appendages in order to avoid variations due to bilateral asymmetry. The 54 precopula pairs collected, and a sample of 50 males and 50 females randomly chosen from the pool of unpaired individuals of reproductive size (appraised by the body size of the smallest male and female in precopula pairs), were retained for shapes analysis. Carpos were removed from the paraeopods in order to access the whole outline of these structures. In the same way, pleotelsons were isolated from the rest of the body. For all characters, the studied outlines correspond to the two-dimensional projection of the dorsal view. They were carefully computer-drawn from numeric photographs.

FOURIER ANALYSIS OF OUTLINES

Most of the methods for outline analyses consist in expressing outlines in periodic signals. Using Fourier transform, such signals are then fitted by a sum of trigonometric functions (or harmonics) that have different amplitudes and phases. Generally, periodic signals are produced by considering the x and y cartesian coordinates of N sampled points on the boundary. The complex notation ($z = x + iy$) of the coordinate pairs is advantageous because it enables the presentation of x and y Fourier transforms in a more compact and elegant form than if they are fitted separately (Moellering & Rayner, 1981). For this reason, we defined a periodic complex series z_n using the complex pairs of 256 points equally spaced on the outline:

$$z_n = x_n + iy_n \text{ with } n \text{ varying from 0 (the starting point) to 255.}$$

Since outlines were described from a finite number of their sampled points and not by a continuous function, discrete Fourier transforms (DFT) were applied to decompose the complex discrete series. Each harmonic is defined from the 256 complex pairs and Z_k, the coefficient of the k harmonic of this Fourier decomposition is:

$$Z_k = \frac{1}{256}\sum_{n=0}^{255} z_n e^{\frac{-i2\pi kn}{256}}$$

The DFT of a periodic series with 256 points is composed of 256 harmonics (k ranging from 0 to 255) and the modulus of Z_k corresponds to the amplitude of k harmonic:

$$A_k = |Z_k|.$$

DFT can be applied to complex series as well as to real series (that is when the imaginary part is fixed to 0). This method allows the description of any outline with a finite number of harmonics. Generally, a shape is adequately described by few harmonics (Crampton, 1995), and, in the case of a complex series, an accurate description is ensured by the first ones and by their conjugates where the harmonic $256 - k$ is, for $k \geq 1$, the conjugate of the harmonic k (Moellering & Rayner, 1982; Kincaid & Schneider, 1983). A reconstruction of any outline can be performed using the formula for discrete inverse Fourier transforms. For each point, a reconstruction can be obtained by summing its complex pairs estimated from all harmonics or by summing its complex pairs calculated from the few harmonics that correctly describe the outline. The complex pair of the point n, calculated from the P retained harmonics is:

$$z_n = \sum_{k=1}^{P} Z_k e^{\frac{i2\pi kn}{256}}$$

Shape variations were studied using amplitudes of the harmonics. In order to consider shape alone, all amplitudes were standardized in size by dividing them by the square root of the character area. The zero harmonics were removed from analyses since they are dependent on translations. The number of harmonics required to ensure a satisfactory fit to outlines was appraised from the Fourier power spectrum (Crampton, 1995). For all studied characters, harmonics 1–3 and their conjugates (that is harmonics 255–253) accounted together for more than 99% of the total power. Because the set of intermediate harmonics had a relatively low contribution to the amount of shape information (less than 1%), amplitudes of these harmonics (harmonics 4–252) were excluded from the statistical analyses. Coordinate extractions and calculations were performed using the software CDFT 2.7 (Dommergues, 2001) developed on MATLAB 5.2.

MEASUREMENT ERROR

Many factors such as light or operator-error in the positioning of characters during the photograph sessions could affect the accuracy of measurements. In order to estimate the reproducibility of our measures, a second repeated measurement was performed on a random sample of 30 specimens (15 females and 15 males) for each character. Measurement error was estimated on the standardized amplitudes of the six retained harmonics as the proportion of the total variance attributable to within-individual variation (Bailey & Byrnes, 1990). Percentages of measurement error associated with the shape measures of the pleotelson and paraeopods 4 and 5 (Table 1) were acceptable for such an intraspecific study since the majority of them were less than 10%. In contrast, percentages of measurement error estimated for head and the paraeopod 1 were rather important since the variabil-

Table 1. Percentage of measurement error associated with the five studied characters. Measurement errors were calculated on standardized amplitudes of the six retained harmonics as the proportion of the total variance attributable to within-individual variation

	Harmonics					
Character	A1	A2	A3	A253	A254	A255
Head	9.37	32.17	25.11	13.21	57.98	3.28
Paraeopod 1	0.39	25.67	37.35	0.51	47.38	0.21
Paraeopod 4	3.69	4.42	14.77	7.79	3.81	0.98
Paraeopod 5	1.05	18.65	9.78	1.42	10.07	0.33
Pleotelson	1.20	1.26	6.03	8.49	3.39	2.34

ity within individuals can be higher than the variability between specimens (amplitude 254 for head, Table 1). Hence, we chose to exclude these two characters from our study.

Measurement error was also appraised on body size. To do this, all collected specimens were measured twice and the percentage of measurement error was estimated to account for less than 0.1%.

SEXUAL DIMORPHISM

Differences in shape between males and females were illustrated by principal components analyses (PCA) performed on the standardized amplitudes of the six retained harmonics. Since males are larger than females in *A. aquaticus* (Steel, 1961; Balesdent, 1964), it was required to control for the possibility that sexual dimorphism in shape is simply an allometric consequence of the sexual body size dimorphism. Therefore, shape dimorphism was assessed, for each character, using multivariate analyses of covariance (MANCOVA) on standardized amplitudes (dependent variables) with sex as the main effect (i.e. males vs. females) and body size as the covariate. Because homoscedasticity of variances was not always fulfilled, we used Pillai's trace for testing significance of MANCOVAs.

COMPARING PAIRED AND UNPAIRED MALES

We compared body size between paired and unpaired males since differences in mean size in relation to pairing status have been reported in *A. aquaticus* (Steel, 1961; Ridley & Thompson, 1979). Variations in shape between both categories of males were investigated with the same methodology used to assess sex differences in shape.

Since apparent differences in the shape of a character between the two categories of males might be induced by correlations between traits (Lande & Arnold, 1983; Endler, 1986; Bell, 1997), we also examined the patterns of covariations between the shapes of the different studied characters for both paired and unpaired males. In order to do this, we used scores on the first principal axis (PC1) of the PCA performed for males on the retained six standardized amplitudes as shape variables. The degree of association between the shape of characters was assessed from the partial correlation of their scores on PC1 adjusted for body size.

VISUALIZING SHAPE VARIATION

An average shape (consensus) was calculated for each character and for the different groups of interest. These consensus may help to detect which parts of the studied structures differ between males and females and between paired and unpaired males, but they do not account for the overall differences demonstrated through MANCOVAs. Consensus were generated from all the reconstructions (obtained with the six retained harmonics) using the Generalized Least-Square (GLS) Procrustes superimposition method (Rohlf & Slice, 1990; Rohlf & Marcus, 1993). While this method is generally used to compare landmarks configurations, it also allows the comparison of a sequence of semi-landmarks that are evenly distributed along outlines provided that terminal landmarks of the sequence are homologous (Bookstein, 1997 in Pavlinov, 2001). Since a precise starting point had been defined for each character, this method was appropriate for our purpose.

The GLS Procrustes superimposition method involves three successive steps: translations in order to superpose centroids of all objects, scaling of the configurations centroid size to unity and rotation to minimize the overall distance between landmarks (Rohlf & Marcus, 1993). The consensus is calculated such that its landmark configuration minimizes the summed squared of landmark distances (so called Procrustes distance) for all studied configurations (Slice *et al*., 1996).

RESULTS

SEXUAL DIMORPHISM

We found extensive sexual dimorphism in our sample of *A. aquaticus*. PCA assessed on standardized amplitudes illustrated these overall morphological dimorphisms (including morphological differences between sexes due to the effects of body size dimorphism). In all cases, male and female groups were clearly separated for all studied characters (Fig. 2) on the first principal axis (PC1), which summarized an important amount of the morphological variability. The separation is particularly strong for paraeopods 4 and 5. For pleotelson, the two groups overlapped partly on PC1. No striking differentiation between males and females appeared on PC2 for any character (Fig. 2).

The MANCOVAs revealed that the shapes of the three characters were allometrically related to body size since effects of body size were always significant (Table 2). In addition, allometric relationships of males were different from those of females, the significant interaction terms demonstrating differences in allometric slopes between sexes (Table 2). Thus, the larger male body size compared to female size (*t*-test: $t = -18.28$, 216 d.f., $P < 0.0001$) was not exclusively responsible for the observed morphological dimorphisms, and male and female shapes differed even when body size was held constant.

Representations of the mean shapes revealed that the most striking morphological dimorphism concerned the carpos of the fourth pair of paraeopods (Fig. 3). For this character, differences in curvatures produced a more convex anterior margin for males than for females (Fig. 3A). Similarly, the concavity of the median posterior region was more pronounced in males (Fig. 3A). The only region of the carpos that was not dimorphic was the proximal posterior area that allows the insertion of the carpos in the meros (Fig. 3A).

On the carpos of the fifth pair of paraeopods, only the proximal anterior and distal posterior areas were dimorphic (Fig. 3B). These patterns of dimorphism were, to a much lesser extent, the same as the ones described above for the fourth pair of paraeopods (Fig. 3B).

Morphological dimorphism displayed by the pleotelson was weak. The posterior region of females' pleotelson seemed to be slightly more concave than the pleotelson of males (Fig. 3C).

Table 2. Results of the MANCOVA performed on the standardized amplitudes of the six retained harmonics for each character (Df Num. and Df Den. indicate the degree of freedom of the numerator and denominator, respectively)

	Paraeopod 4				Paraeopod 5				Pleotelson			
Effect	Df Num.	Df Den.	Pillai's trace	*F*	Df Num.	Df Den.	Pillai's trace	*F*	Df Num.	Df Den.	Pillai's trace	*F*
Model	18	504	1.400	24.519***	18	474	1.069	14.577***	18	435	1.124	14.471***
Sex	6	166	0.147	4.756***	6	156	0.288	10.511***	6	143	0.221	6.779***
Body size	6	166	0.491	26.695***	6	156	0.677	54.568***	6	143	0.701	55.957***
Sex*Body size	6	166	0.144	4.669***	6	156	0.191	6.135***	6	143	0.218	6.638***

***$P < 0.001$.

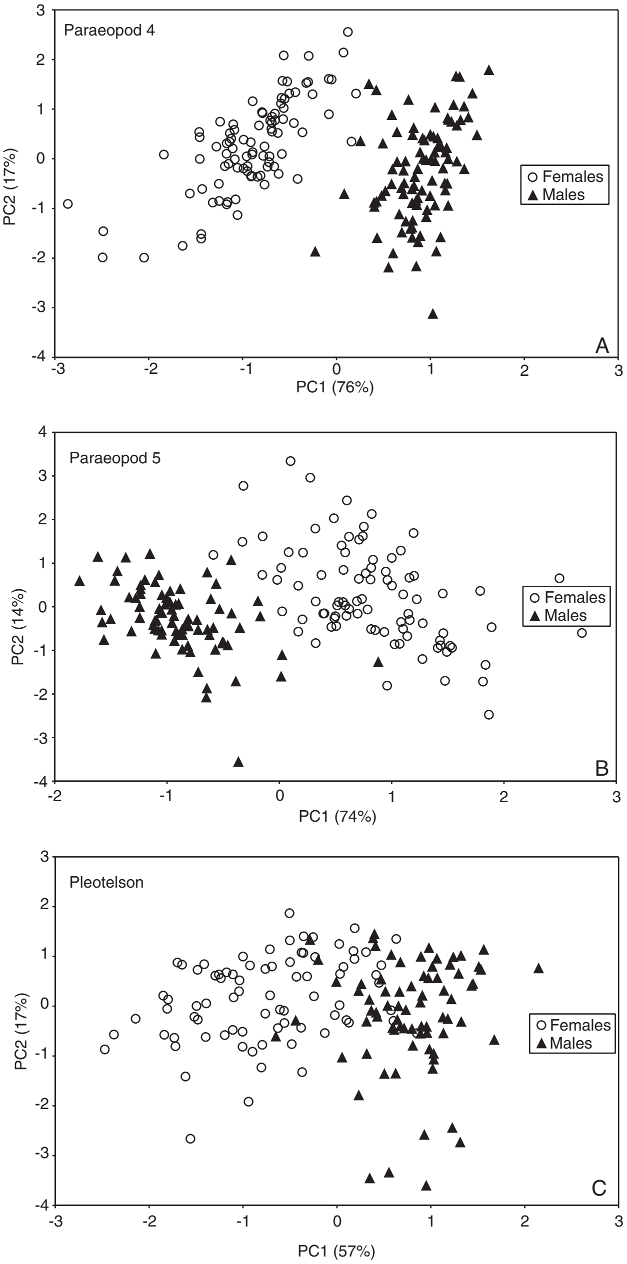

Figure 2. Plot of the scores of males and females for the principal component analyses performed on the standardized amplitudes of the six retained harmonics for paraeopod 4 (A), paraeopod 5 (B) and pleotelson (C).

COMPARING PAIRED AND UNPAIRED MALES

Paired males were significantly larger than unpaired ones (*t*-test: $t = -2.776$, 101 d.f., $P < 0.01$), supporting previous observations (Steel, 1961; Ridley & Thompson, 1979). The MANCOVAs performed on the standardized amplitudes of the six retained harmonics indicated variations in shapes between the two cat-

Table 3. Results of the MANCOVA performed on the standardized amplitudes of the retained harmonics for each character (Df Num. and Df Den. indicate the degree of freedom of the numerator and denominator, respectively)

	Paraeopod 4				Paraeopod 5				Pleotelson			
Effect	Df Num.	Df Den.	Pillai's trace	*F*	Df Num.	Df Den.	Pillai's trace	*F*	Df Num.	Df Den.	Pillai's trace	*F*
Model	18	243	0.726	4.311***	18	216	0.814	4.466***	18	198	0.944	5.050***
Pairing status	6	79	0.185	2.986*	6	70	0.069	0.867	6	64	0.200	2.661*
Body size	6	79	0.534	15.077***	6	70	0.615	18.650***	6	64	0.638	18.817***
Pairing status*Body size	6	79	0.171	2.716*	6	70	0.069	0.860	6	64	0.180	2.3488*

***$P < 0.001$; *$P < 0.05$.

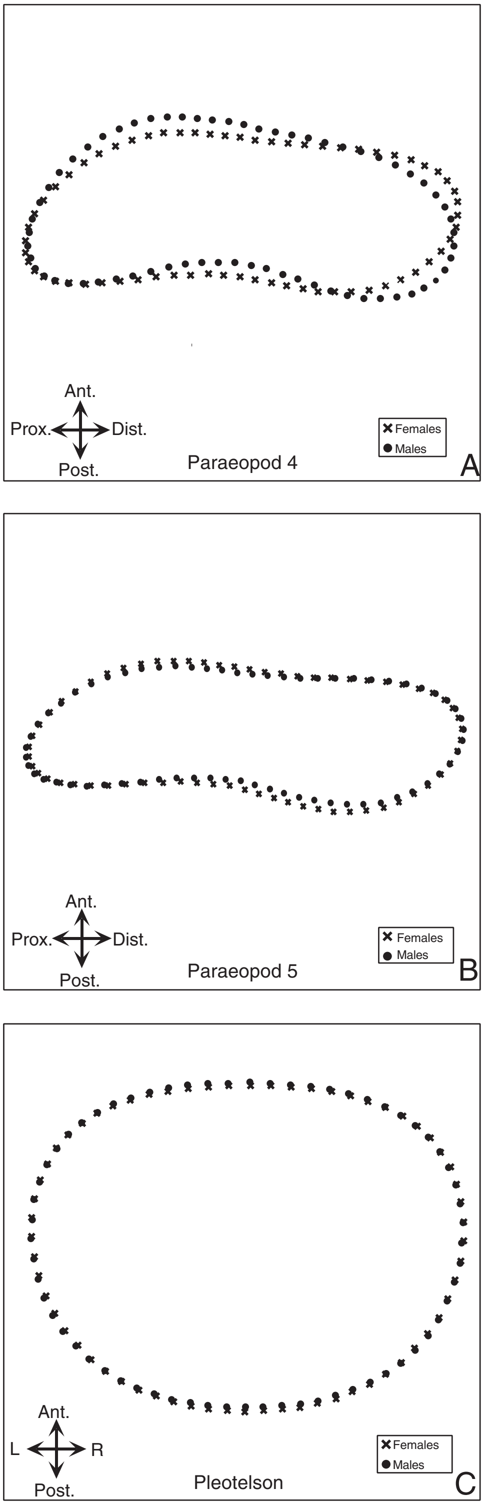

Figure 3. Average shape (consensus) of males and females calculated from all the reconstructions with six harmonics for paraeopod 4 (A), paraeopod 5 (B) and pleotelson (C). To improve the clarity of the figures, only 64 points of these consensus are represented instead of the 256 used for calculations.

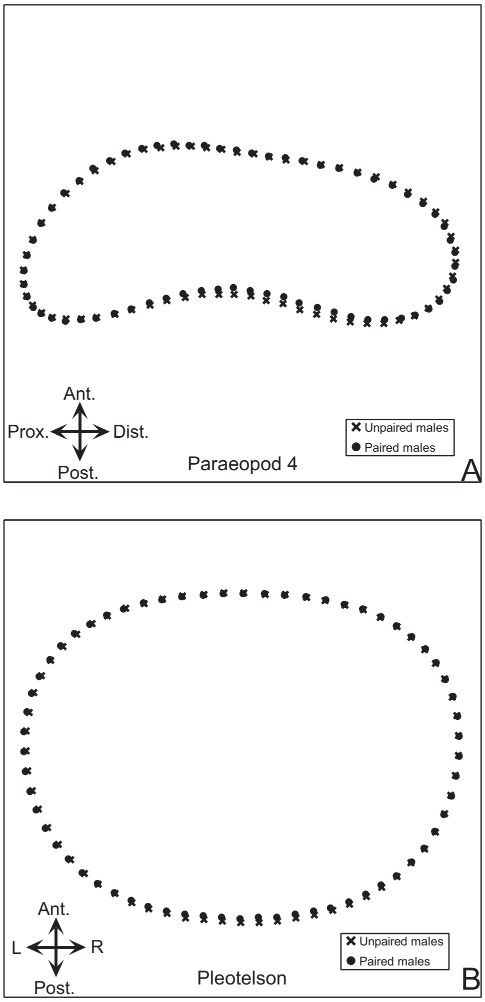

Figure 4. Average shape (consensus) of paired and unpaired males calculated from all the reconstructions with six harmonics for paraeopod 4 (A) and pleotelson (B). To improve the clarity of the figure, only 64 points of these consensus are represented instead of the 256 used for calculations.

egories of males (Table 3). For paraeopod 4 and pleotelson, allometric relationships between shapes and body size were significantly different between paired and unpaired males (Table 3). In contrast, for the fifth pair of paraeopods, allometric relationships were identical for the two categories of males since neither the allometric slopes nor the intercepts (pairing status effect) differed between paired and unpaired males for this character (Table 3) and, for intercepts, even when the interaction term was removed from the model. There was no divergence in the shape of this character when body size was held constant between paired and unpaired males.

Unsurprisingly, consensus representations indicated that morphological differences between the two

Table 4. Correlation coefficients between the shape of paraeopod 4, paraeopod 5 and pleotelson for paired and unpaired males. The residuals obtained from regressing PC1 scores on body size for both paired and unpaired males were used as shape variable (values in parentheses correspond to samples sizes)

Pairing status	Paraeopod 4 Paraeopod 5	Paraeopod 4 Pleoteson	Paraeopod 5 Pleotelson
Paired Males	–0.01 (42)	0.49* (33)	–0.11 (30)
Unpaired Males	0.05 (33)	0.08 (34)	–0.17 (31)

**P* < 0.05 after Bonferroni correction for risk level on the table.

categories of males were weaker than those observed for sexual dimorphism (Figs 3,4). However, it is worth noting that differences in the shapes of the paraeopod 4 and the pleotelson between paired and unpaired males only concern regions that were described as sexually dimorphic in the previous section. Thus, the concavity of the median posterior region of the carpos of the fourth pair of paraeopods was more pronounced in paired males (Fig. 4A). In the same way, unpaired males seemed to have a more concave posterior margin of the pleotelson than did the paired males (Fig. 4B).

With regard to the covariation of the characters, when body size was held constant, the only significant correlation between the shapes of characters concerned paraeopods 4 and the pleotelson of paired males. All the correlations calculated for the other combinations of characters were not significant for both categories of males (Table 4).

DISCUSSION

Two previous studies (Vandel, 1926; Balesdent, 1964) reported strong sexual dimorphism in *A. aquaticus* for the first and fourth pair of paraeopods. However, both studies relied exclusively on a qualitative approach. Here, using a quantitative approach, we confirm earlier observations for pareopods 4 and demonstrate that sexual dimorphism in *A. aquaticus* concerns at least two others characters: the pleotelson and the paraeopods 5. Beyond their efficiency for intraspecific studies (Lestrel, Sarnat & McNabb, 1989; Lestrel *et al*., 1993; Loy, 1996; Roth & Mercer, 2000), Fourier methods now appear to be powerful tools for unravelling subtle morphometric differences. However, it is worth noting that, at this level of comparison, the measurement error, estimated as the proportion of the total variance attributable to intra-individual differences, increases. This was probably the case for the propodos of the paraopods 1 and for the head, for which measurement error inhibited our ability to quantify shape variation.

Shape differences between male and female *A. aquaticus* were established for all the three characters retained, two of them, the pleotelson and the paraeopods 5, having never been described as dimorphic structures. This result suggests that the divergence between sexes affects the shape of numerous characters and raises the question of their adaptive significance. Until now, male–male competition has been regarded as the major determinant for the evolution of sexual dimorphism in *A. aquaticus*. Indeed, sexually dimorphic characters such as paraeopods 1 and 4 are used by males to grasp females during mate guarding (Vandel, 1926; Balesdent, 1964). In addition, hypogean Asellidae species, in which copulation is not preceded by mate guarding, do not display such sexual divergences (Henry, 1976). Our study provides new arguments that support an influence of precopulatory mate guarding in shaping male morphology in *A. aquaticus*. Indeed, the significant differences found between paired and unpaired males for the shape of paraeopods 4 and pleotelson suggest that sexual selection, through males' pairing advantage, could be partly responsible for the maintenance of the sexual dimorphism within these two characters. Moreover, shape differences between the two categories of males are restricted to regions of the characters that are sexually dimorphic, with paired males showing the most extreme divergences from females. Since unpaired males can displace smaller males to take over females (Ridley & Thompson, 1979), the reproductive success of males may partly depend on the ability to withstand such attempts. Overcoming female resistance to male guarding attempts may also influence a male's pairing success. Female resistance has been documented in many mate guarding species as a result of intersexual conflicts in guarding duration (for review in amphipod and isopod crustaceans see Jormalainen, 1998). However, the role of female behaviour in pair formation in

A. aquaticus remains controversial. Female resistance was observed by Ridley & Thompson (1979) but was considered as negligible in other studies (Manning, 1975; Jormalainen & Merilaita, 1995). In such a context, the greater curvature of the fourth pair of paraeopods may benefit males by enhancing their grasping ability. How the differences in pleotelson morphology might confer a reproductive advantage is unclear since no implication of this character in mate guarding has ever been reported. Therefore, direct observations of sexual interactions are then required to test if pleotelson consistently plays a role in pair formation or whether its dimorphism arose as the result of a correlational response to selection for the shape of paraeopods 4 or body size (Lande & Arnold, 1983; Endler, 1986; Bell, 1997). A strong underlying genetic correlation of the shapes between the fourth pair of paraeopods and pleotelson might be excluded since no association between the shape of these characters was demonstrated for unpaired males. However, sexual selection probably favours a combination of these character states since such a relationship was detected for paired males.

Interestingly, sexual shape dimorphism was also demonstrated for a character that is not different between paired and unpaired males: the paraeopod 5. This result could indicate that mate guarding might not be the only force that entails sexual dimorphism in *A. aquaticus*. Morphological divergences between the two sexes might be induced by differences between males and females in reproductive roles. For instance, since males actively seek for mates during the breeding season whereas females do not (Vandel, 1926; Balesdent, 1964), males could display specific characteristics that enhance their walking performance. We might expect that such characteristics concern paraeopods because of their obvious role in locomotion. Further investigation of the link between female encountering rate, walking performance and the shape of paraeopods 5 should help to clarify this issue.

Until now, in the vast majority of invertebrate species including *A. aquaticus*, attention has mainly been paid to relationships between body size and mating success (Ridley & Thompson, 1979; Ward, 1988; Jormalainen, Tuomi & Merilaita, 1992; Carroll & Salamon, 1995; Savalli & Fox, 1998). However, because of correlations between quantitative traits, a mating advantage of large males may arise even if the actual target of sexual selection is not the overall body size *per se* but actually the size or shape of a particular morphological trait. Given allometric relationships between shape and body size, multivariate analyses of selection would then be required to determine which characters undergo direct sexual selection (Lande & Arnold, 1983). Thus, the combined use of a Fourier method and multivariate analyses of selection may provide powerful tools to investigate how morphology affects reproductive success, and hence to broaden our understanding of sexual dimorphisms, particularly in relation to the geometry of structures.

ACKNOWLEDGEMENTS

We thank Cyril-Hugues Dommergues for providing us the software CDFT 2.7. Stewart Plaistow and two anonymous referees made valuable comments on the manuscript. A. Bertin was supported by a doctoral grant from the Ministère de la Recherche. This work was in part funded by a 'Contrat d'accueil des nouveaux chercheurs, 3ème contrat de Plan Etat-Région Bourgogne' to P. Alibert. It is a contribution of the themes 'Ecologie évolutive' and 'Signal morphologique de l'Evolution' of the UMR CNRS 5561, and of the GDR 2474 CNRS Morphometrie et Evolution des Formes.

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