Hydrobiologia **220**: 89–98, 1991.

Shape variation in the Australian freshwater mussel *Alathyria jacksoni* Iredale (Bivalvia, Hyriidae)

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Received 5 October 1989; in revised form 23 October 1990; accepted 25 November 1990

Abstract

Different forms of the hydriid mussel *Alathyria jacksoni* occur in riverine habitats of the Murray-Darling river system, south-eastern Australia. Electrophoretic data for three enzymes (glucose phosphate isomerase, malate dehydrogenase and malic enzyme) support the assumption that these are morphological variants of one species. Shells from the Murray in South Australia are elongate-oval, with the dorsal margin extended posteriorly as a blade or 'wing', whereas shells from Victoria and New South Wales have a dorsal arch and a postero-ventral inflection. The variation appears to be associated with water velocity. The arched shells have comparatively large, strong adductor muscles; the posterior adductors are elongate and both anterior and posterior adductors are displaced ventrally. These internal modifications are reflected in changing shell shape as size increases. The changes apparently enhance the mussel's ability to maintain an anchorage in a strong current, but lessen the effectiveness of its valve seal. An incidental observation is that the related species *A. condola* Iredale, known from the Murrumbidgee and Lachlan rivers, may also occur in the middle reaches of the Murray.

Introduction

Molluscs show the adaptive impress of their local environment more clearly than other, more mobile animals (Morton, 1967). In particular, shell shape varies markedly in the freshwater mussels of the Unionacea, found in lakes and rivers throughout the world. Many attempts have been made to discover systematic relationships between unionid shell shape and the environment (e.g. Ortmann, 1920; Ball, 1922; Agrell, 1948; Kauffman, 1969; Green, 1972), but without spectacular success. More recent work on marine bivalves, concerned with functional analysis rather than description and correlation, suggests that shell and body are a functional unit and should not be considered in isolation (e.g. Kauffman, 1969; Stanley, 1970). This integrated approach has yet seen little application in regard to freshwater mussels.

The freshwater pearl mussel of the Northern Hemisphere (*Margaritifera margaritifera* L.) shows a striking dual pattern of shape variation in that shells from strong currents develop a strong dorsal arch and a ventral inflection (Altnoder, 1926). Eagar (1948) drew attention to this, and to similar variation shown by fossil anthracosiid bivalves. He later ventured an explanation for the adaptive significance of the variation (Eagar, 1978), incorporating earlier work by Trueman (1966, 1968). Eagar proposed that arching modified the hinge structure in such a way that the pedal gape was increased, allowing the foot to

extend further into the sediment and gain a stronger anchorage in fast-flowing water.

Walker (1981) observed similar variation in the hydriid mussel *Alathyria jacksoni* Iredale from the middle and lower reaches of the River Murray, south-eastern Australia. Shells from the middle reaches (e.g. Echuca to Mildura, Victoria) typically have an arched dorsum, whereas those from the lower reaches (e.g. Renmark to Morgan, South Australia) have the dorsum extended backward as a blade or 'wing' (Fig. 1). The concentration of weirs along the lower river, and casual observations, suggest that the middle reaches are subject to stronger currents than the lower reaches; the 'arched' from of *A. jacksoni* therefore appears to occur in faster-flowing water than its 'winged' counterpart (see further Walker, 1985, 1986; Sheldon & Walker, 1989).

Here, we explore further the nature of shape variation displayed by *A. jacksoni*. First, electrophoretic and other data are presented to support morphological evidence that the two forms are

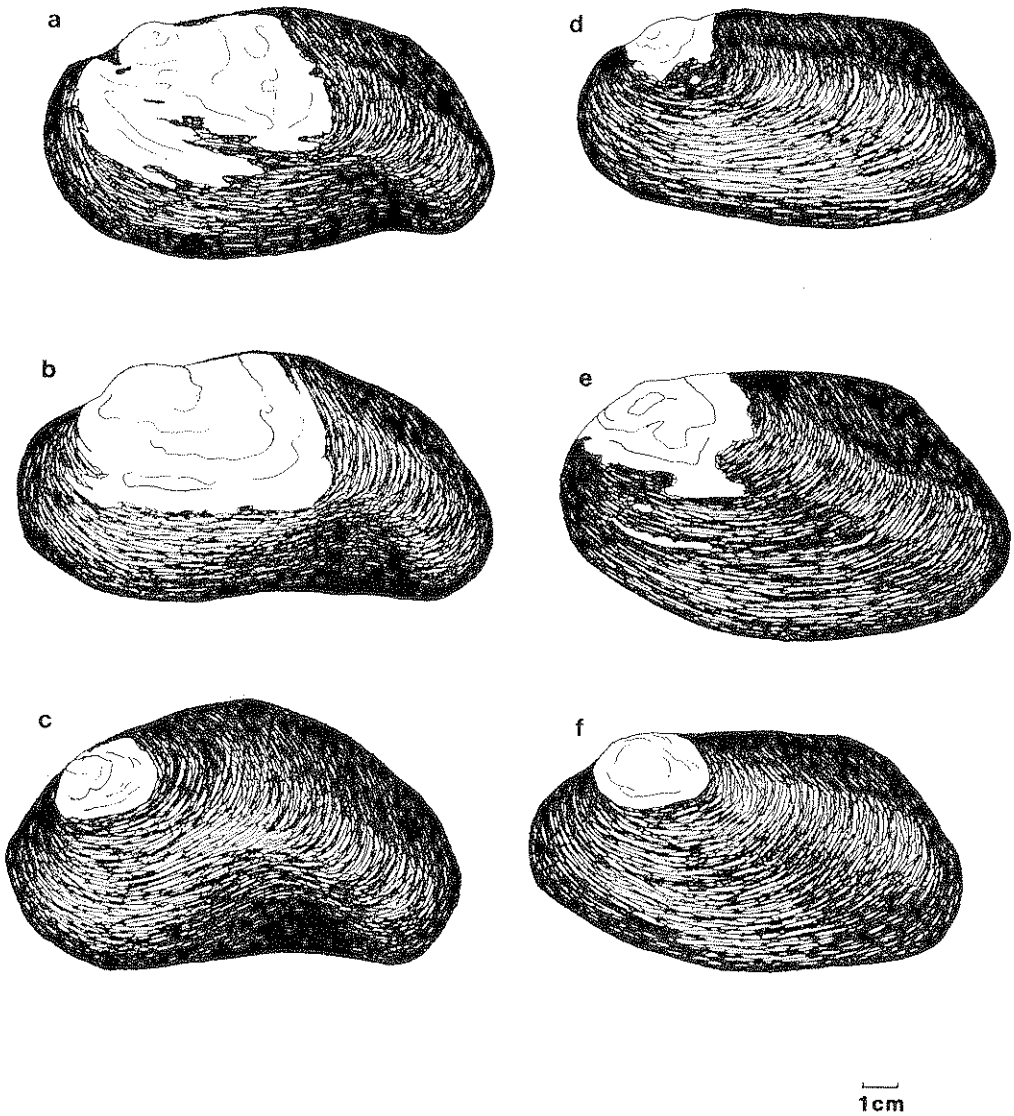

Fig. 1. Shell shape variation in *Alathyria jacksoni*: a–c arched form; d–f, winged form.

members of the one species. This is important because the taxonomy of hyriid mussels is founded upon shell morphology, despite wide variability within and between populations (McMichael & Hiscock, 1958). Davis (1983) found that analysis of protein variation provides important evidence for assessing relationships among molluscan taxa. In addition, there is some risk of confusion between *A. jacksoni* and *A. condola* Iredale, a species known from the Murrumbidgee and Lachlan drainages (Walker, 1981) and, as this paper will show, probably also present in the middle reaches of the Murray. Second, the extent of shape variation in *A. jacksoni* is described by physical measurements of shell and body features. Finally, we speculate about the functional significance of the variation.

Abbreviations used in the text: N.S.W. New South Wales, S.A. South Australia and Vic. Victoria.

Materials and methods

Electrophoretic investigations

Mussels were collected by diving and transferred within 6 h to well-aerated laboratory aquaria with sand substrata. Samples were obtained from the Mulwala Irrigation Canal and the River Murray below Lake Mulwala (N.S.W.), at Boundary Bend and Wemen (Vic.) and at Lock 2 (Waikerie), Morgan, Walker Flat and Blanchetown (S.A.). In addition, samples were collected of *A. jacksoni* at Lock 3 (R. Murray at Overland Corner, S.A.) and *A. condola* at Darlington Point (Murrumbidgee R., N.S.W.); these had shell characters typical of their nominate species (cf. McMichael & Hiscock, 1958) and were used as 'types'.

Cellulose acetate gel electrophoresis was employed to make comparisons between the 'types' and other samples. Tissues for analysis were cut from the foot region (0.5 ml of muscular, digestive, reproductive and mantle tissue). The tissue was macerated and treated with 1.0–1.5 ml lysing buffer (0.1 ml non-ionic detergent TRITON × 100, 0.1 ml B-mercaptoethanol, 10 mg NAD and 10 mg NADP in 100 ml 20 mM HEPES buffer). The lysate was centrifuged at 8000 rpm for 25 min at 4 °C, and samples were immediately applied to Cellogel 500 (Chemetron, Italy) in an electrophoresis tank containing 400 ml buffer (Tris-EDTA-borate, pH 7.8). Gels were run for 2 h on a 200-V DC, 10–15 mA power supply, then placed in a 2 ml enzyme-specific stain solution prepared from dry ingredients less than 1 min before use. When bands had attained maximum definition the reaction was terminated by immersing the gel in 10% formalin. Gels were then transferred to 20% glycerol, dried and stored between sheets of paper.

From an initial survey of 18 enzymes in single 'type' specimens of *A. jacksoni* and *A. condola* (Balla, 1984), three enzymes were selected for further study. These were glucose phosphate isomerase (stain ingredients: 0.004 g MTT, 0.002 g PMS, 0.002 g NADP, 0.004 g fructose-6-phosphate, 0.1 ml 0.2 M $MgCl_2$, 0.4 ml 0.1 M Tris-HCl pH 8.0 and 12 µl glucose-6-phosphate dehydrogenase), malic enzyme (1.0 ml 0.1 M Tris-HCl pH 8.0, 0.2 ml L-malic acid 2 M pH 8.0 (NaOH), 0.2 ml 0.3 M $MgCl_2$, 0.01 g NADP, 0.005 g PMS, 0.02 g MTT) and malate dehydrogenase (1.0 ml 0.1 M Tris-HCL pH 8.0, 0.2 ml L-malic acid 2 M pH 8.0, 0.2 ml 0.3 M $MgCl_2$, 0.01 g NAD, 0.02 g MTT and 0.005 g PMS).

Functional analysis

Shell shape was measured with calipers (Mitutoyo, accurate to 0.05 mm) using a series of linear measurements designed to highlight the differences between forms (Fig. 2). Calipers were also used to measure the distance between the anterior and posterior adductors and the dorsal-ventral length of the posterior adductor (Fig. 2). The cross-sectional areas of the adductors were estimated by measuring their dorso-ventral and antero-posterior radii and applying the standard formula for the area of an ellipse.

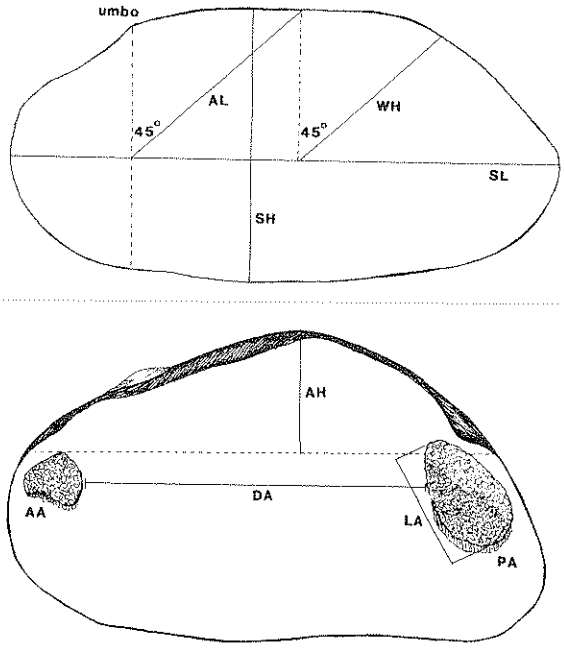

Fig. 2. Shell shape and internal measurements referred to in the text. AA anterior adductor area; AH arch height, AL arch length, CA combined adductor area, DA distance between adductors, LA length of posterior adductor, PA posterior adductor area, SH shell height, SL shell length, WH wing height.

To determine the strength of the adductor muscles a series of measurements was made with the mussels removed from water, to provoke them into valve closure. A section of polyethylene tubing (external diameter 16.0 mm, compressible to 4.0 mm) was inserted mid-ventrally between the valves. Using calipers, the gape between the valves was measured at 15 min intervals for 1 h, then at 30 min intervals for a further 3 h. As the gape generally varied less than 1 mm over the 4 h period, only the data for the first hour are reported here. A Salter 0–2 kg spring balance was used to correlate weight with tube compression (Balla, 1984). The area compressed by the bivalve shell was approximately 1 cm^2. Pressure is a measure of force per unit area and force is a measure of weight and acceleration. If the acceleration of this weight is assumed to be due to gravity alone, then 1 kg force is 9.80665 N and pressure = X (kg) $\times$ 9.80665/1 $\times$ 10^{-4} Nm^{-2}.

Results

Electrophoretic investigations

Of 18 enzymes initially tested by Balla (1984), five produced no visible bands (viz. acid phosphatase, fumerase, glucose-6-phosphate dehydrogenase, nucleotide phosphorylase and superoxide dismutase). Others indicated no variation between *A. jacksoni* and *A. condola* (viz. alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, glutamate oxaloacetate transaminase, lactate dehydrogenase, peptidase A (two alleles) and mannose phosphate isomerase). The effective discriminators were adenylate kinase (bands very pale), peptidase B (pale) and 6-phosphogluconate dehydrogenase (pale), glucose phosphate isomerase, malic enzyme and malate dehydrogenase. The last three were chosen for further study because (a) they require common stain ingredients, so minimizing the cost of analysis, (b) each is part of an important energy-producing pathway and (c) the first two are regulatory enzymes and should be particularly sensitive to selective forces (cf. Johnson, 1974).

There are obvious similarities between the different samples of *A. jacksoni* in respect to glucose phosphate isomerase (Fig. 3) and malate dehydrogenase and malic enzyme (Fig. 4). This is some evidence to regard them as members of the one species.

The putative 'forms' of *A. jacksoni*, from a variety of localities, were easily distinguishable from *A. condola* using glucose phosphate isomerase: *A. jacksoni* consistently indicated one allele, whereas *A. condola* showed as many as three distinct alleles (Fig. 5). Of a total of 32 individuals 0% were homozygous for allele a, 37.5% were homozygous for allele b, 3.12% were homozygous for allele c, 15.63% were a–b heterozygous and 43.75% were b–c heterozygous. Other differences between *A. jacksoni* and *A. condola* were evident from tests with malate dehydrogenase and malic enzyme (Fig. 4). These results suggest that *A. jacksoni* is electrophoretically distinct from *A. condola*.

An unexpected result is that the bands for some

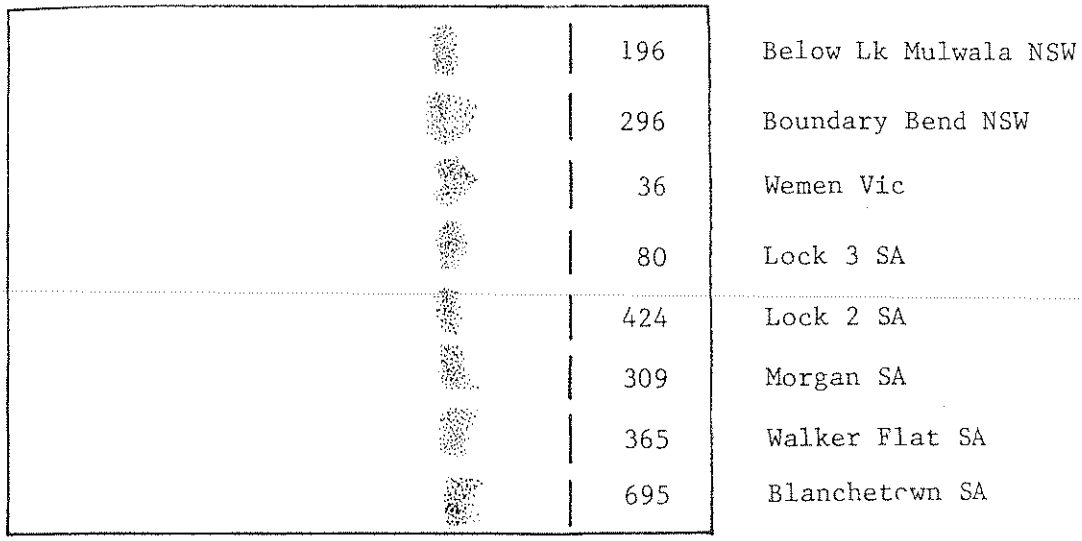

Fig. 3. Electrophoretic bands for glucose phosphate isomerase for *Alathyria jacksoni*.

Balla (1984), five
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presumed specimens of *A. jacksoni* were like those for *A. condola* from the Murrumbidgee River at Darlington Point (Fig. 4), rather than the bands for 'typical' *A. jacksoni* from Lock 2. This suggests that *A. condola* may occur at several sites along the middle reaches of the Murray, but requires confirmation for more loci.

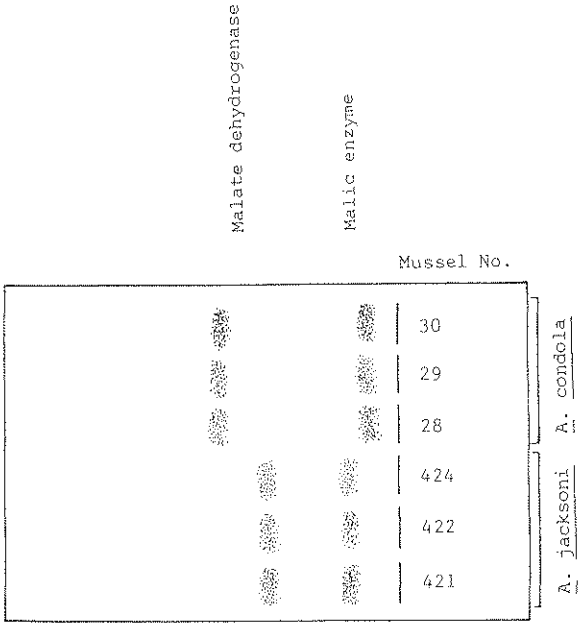

Fig. 4. Electrophoretic bands for malate dehydrogenase and malic enzyme in *Alathyria jacksoni* and *A. condola*.

Functional analysis

Shell-shape measurements (Fig. 2) were made to quantify shape variation in *A. jacksoni*, and internal measurements (adductor strength, size and separation) were made to elucidate the functional significance of the variation. Samples of 24 mussels from a typically 'arched' population (Wemen, Vic.) and 17 mussels from a 'winged' population (Lock 2, Waikerie, S.A.) were compared using Spearman rank correlation coefficients. Analyses of the separate samples showed only one significant correlation: in the 'winged' sample, adductor strength was correlated with combined adductor area ($r = 0.703$, $n = 17$, $P < 0.05$).

Discriminant Analysis (SPSS Inc., 1983) was used to investigate the discriminant score as a possible measure of arch development in the same two samples. Discriminant Analysis forms linear combinations of *discriminating variables* (shell measurements) and *discriminant coefficients* (between and within group sums of squares and cross product matrices) to produce a *discriminant*

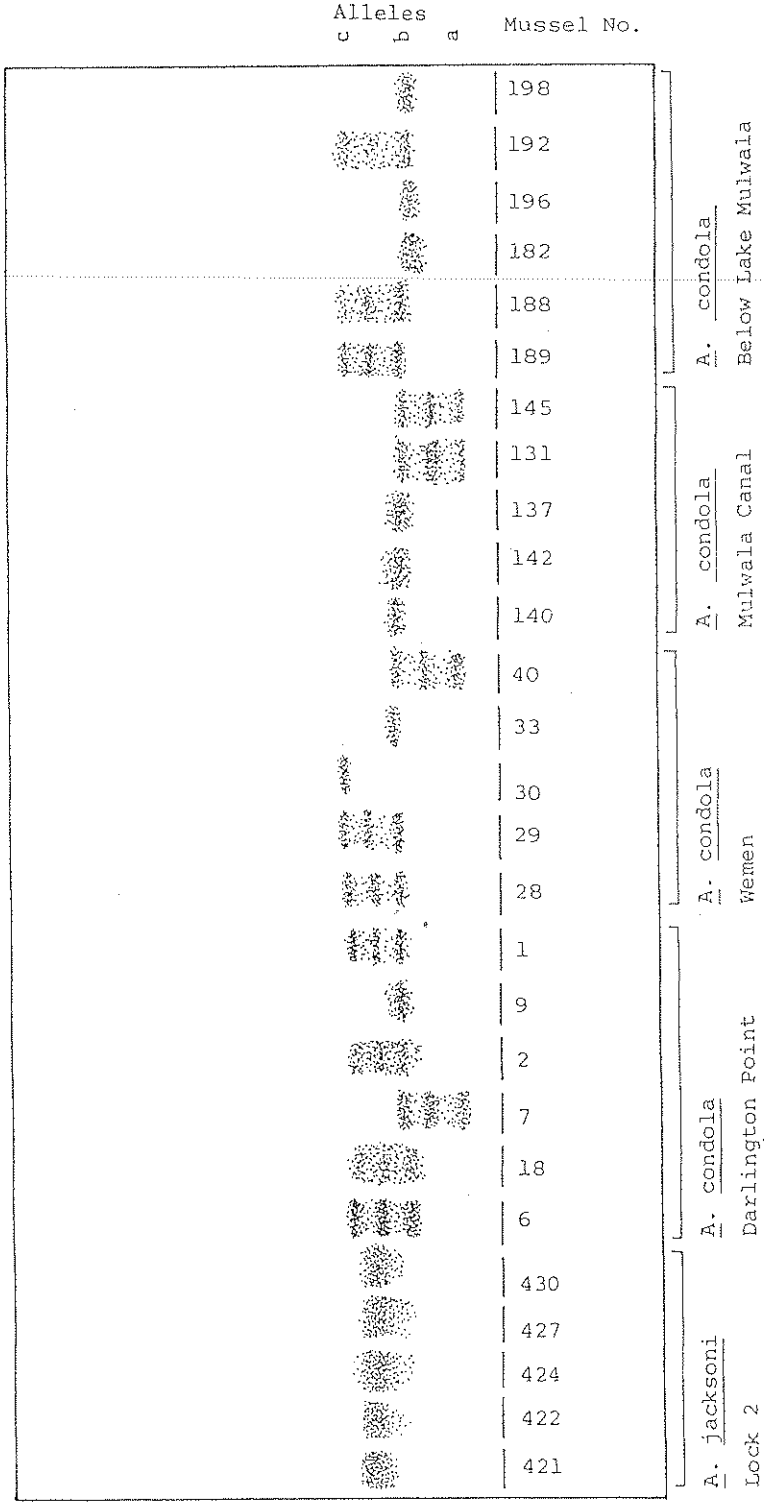

Fig. 5. Comparison of specimens of *Alathyria jacksoni* and *A. condola* from several populations, with regard for glucose phosphate isomerase.

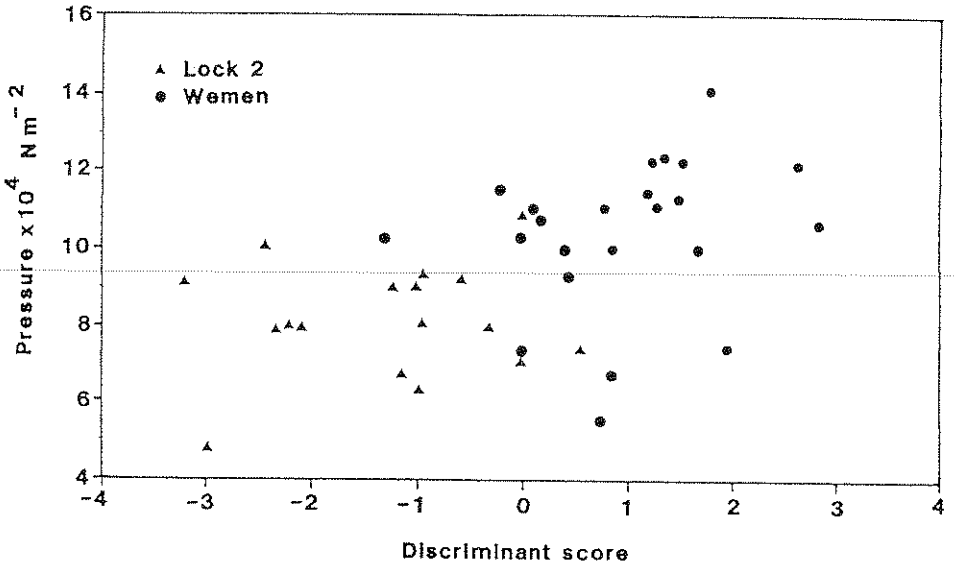

Fig. 6. Discriminant score and pressure (Nm^{-2}) of valve closure as a measurement of adductor strength for specimens of *Alathyria jacksoni* from the River Murray at Wemen (Vic.) and Lock 2 (S.A.).

score. Any single score represents the number of standard deviations that case is away from the mean for all cases on the given discriminant function. A plot of discriminant score *versus* adductor strength is shown in Fig. 6. The results suggest that there is a continuum of arching, and a positive correlation between arching and adductor strength (i.e. individuals) with strong arch development have more powerful adductor muscles). The correlation is supported by direct measurements: the median adductor strength of the 'arched' *A. jacksoni* (10.4×10^4 Pa, $n = 24$) was 30% greater than that of the 'winged' specimens (7.3×10^4 Pa, $n = 17$). Further, the mean adductor strength of the 'arched' mussels was 9.5×10^4 Pa (± 0.7 95% CI; min. 4.8×10^4, max. 12.3×10^4) and that of the 'winged' mussels was 7.5×10^4 Pa (± 0.4; 4.5×10^4, 9.4×10^4).

If the shape variation is regarded as a continuum and the two samples are pooled ($n = 41$), three points emerge (Table 1). First, correlations ($P < 0.05$) are evident among the distance

Table 1. Spearman rank correlation coefficients for measurements on a pooled sample ($n = 41$) of *Alathyria jacksoni* from the River Murray at Lock 2 (Waikerie, S.A.) and Wemen (Vic.). SH shell height, SL shell length, AS adductor strength, PA posterior adductor area, AA anterior adductor area, CA combined adductor area, AH arch height, AL arch length, WH wing height, LA length of posterior adductor, DA distance between adductors. Asterisks indicate correlations significant at $P < 0.05$.

	LA	DA	CA	PA	AA	SH	WH	AL	AH
AS	0.552*	0.686*	0.372*	0.325*	0.503*		0.697*	0.582*	0.408*
AH	0.306*	0.556*	0.233	0.259	0.138	0.555*	0.578*	0.608*	
AL	0.690*	0.834*	0.462*	0.484*	0.627*		0.718*		
WH	0.596*	0.824*	0.361*	0.417*	0.392*				
SL		0.926*							
AA		0.491*							
PA		0.398*							
CA		0.374*							
DA	0.891*								

glucose phosphate

between the adductors and (a) shell length (r_s = 0.926), (b) length of the posterior adductor (r_s = 0.891), (c) arch length (r_s = 0.834) and (d) wing height (r_s = 0.824). Second, the positive correlations between wing height and arch length (r_s = 0.718), and arch length and arch height (r_s = 0.608) suggest that 'wing height' does not accurately measure the degree of wing development, as one would expect arch and wing development to be negatively correlated. The measurements of arch height, arch length and wing height therefore should be regarded collectively as a measure of arching. Third, adductor strength is correlated with (a) wing height (r_s = 0.697), (b) distance between the adductors (r_s = 0.868), (c) arch length (r_s = 0.582) and (d) length of the posterior adductor (r_s = 0.552).

Discussion

Morphological variation in *A. jacksoni* is such that the 'dual growth forms' as described by Walker (1981) should properly be regarded as extremes of a continuum of variation; indeed, the same is likely to apply to variation in *Margaritifera margaritifera* (cf. Eagar, 1978). Although this study has provided partial clarification, some obvious opportunities remain for ecological studies, particularly with regard for the adaptive significance of the variation in shape.

The electrophoretic data, particularly those for glucose phosphate isomerase, provide a clear diagnosis of *A. jacksoni*, notwithstanding the range of morphological variation. Confirmation is required, however, for a greater number of protein loci. As noted earlier, adenylate kinase, peptidase B and 6-phosphogluconate dehydrogenase may repay investigation (Balla, 1984).

The electrophoretic data also provide partial evidence that *A. condola* occurs in the middle reaches of the River Murray. The form of these shells, however, is not typical (cf. McMichael & Hiscock, 1958; Walker, 1981). Shells of *A. condola* from the Murrumbidgee River are large (to 120 mm length) and rounded, with a variable excavation of the dorsal margin anterior to the umbo. The apparent specimens of *A. condola* from the Murray are morphologically indistinguishable from some *A. jacksoni* based on external shell characters. Davis (1983) regards immunological and alloenzyme data as the most valuable information for assessing taxonomic relationships, followed by comparative anatomy and conchology. Until confirmation is obtained, the taxonomic diagnose of both these species should be regarded with caution.

It is reasonable to assume that the functional anatomy of *A. jacksoni* is similar to that of mussels in the family Unionidae. Like the unionids, *A. jacksoni* exhibits a dimyarian condition, having anterior and posterior adductor muscles of approximately equal size (cf. Kauffman, 1969). Typically, when contracted the adductors hold the valves together and oppose the tensional and compressive forces of the ligament. They incorporate a *catch* portion of non-striated muscle and a *quick* portion of striated muscle. The quick portion is involved in burrowing and in rapid valve closure, which affords a defense mechanism and a means of purging the mantle cavity of water and waste. The catch portion is involved in sustained contraction, and keeps the valves closed against the counter force of ligament (Wilkie, 1968). The strength of the adductors when contracted is governed by their positions relative to each other and the extremities of the shell (Kauffman, 1969).

Trueman (1966, 1968) examined the role of the adductor muscles in burrowing. He showed that adduction subjects the fluids contained within the valves to pressure, resulting in the ejection of water from the mantle cavity and blood into the pedal haemocoel. The ejection of water liquefies the sand adjacent to the shell immediately before the retractor muscles contract to pull the shell down to the foot, allowing easier penetration. In addition, the increased pressure in the foot, together with the relaxation of the pedal muscles, causes the food to expand. This must occur before retraction in order to give a firm anchorage, so that the shell can be pulled down. Sustained anchorage is achieved by wedging the foot in the substratum using contraction of the adductors to

maintain the hydraulic pressure in the fluid-filled foot (Trueman, 1968).

The position commonly adopted by *A. jacksoni* is to burrow into the substratum so that about 2 cm of the posterior end of the shell protrudes. This permits filtering activity *via* the siphons, but also requires that the foot be extended to provide anchorage. An increase in the strength of the current may cause localized scouring of the substratum, and the mussel may need to further extend its foot to maintain position. An unburied mussel is liable to be swept downstream if the current is sufficiently strong. Balla (1984) found that a velocity of 0.3 m s^{-1} is sufficient to transport unburied specimens of *A. jacksoni*.

Stronger adductor muscles probably facilitate burrowing and anchorage. Thus 'arched' *A. jacksoni* from strong currents (e.g. Wemen, Vic.) have stronger adductors than 'winged' *A. jacksoni*, from lesser currents (e.g. Lock 2, S.A.). Winged *A. jacksoni* maintain a sufficient anchorage with adductors having a comparatively modest cross-sectional area, but in areas of stronger current, where arched *A. jacksoni* occur, the anchorage is strengthened by (a) an increase in the cross-sectional area of the adductors, (b) greater separation of the anterior and posterior adductors, (c) ventral elongation of the posterior adductor and (d) ventral displacement of the adductors relative to the ligament (hence, increased leverage).

The position of attachment of the adductors to the shell is determined by the mode of growth; the adductors (the posterior more than the anterior) progressively move from their juvenile positions near the umbo toward the anterior and posterior extremities of the shell. The displacement is often shown by concentric lines around the innermost margins of the adductor muscle scars; it probably is achieved by additions of muscle fibres to the leading edge and degeneration of fibres on the inside edge, so that continuous function is maintained. Consequently, the position of the adductors is adaptable and potentially responsive to environmentally-determined mechanical influences.

The shell may be regarded as a mediator between the animal and its physical environment. For example, the shape of arched shells of *A. jacksoni* is determined by ventral displacement of the adductors, as a response to the external current. The shell reflects the form of the body, and its behavioural responses to the environment, through their effects on the mantle, the tissue responsible for secretion of the shell.

Acknowledgements

Our thanks to Lance Lloyd, Sandi Poland and Jane Moller for field assistance and to Dr Terry Schwaner, formerly of the South Australian Museum, for use of electrophoretic materials and equipment.

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