

# Size matters: systematic and ecological implications of allometry in the responses of chironomid midge morphological ratios to experimental temperature manipulations

Brendan G. McKie and Peter S. Cranston

**Abstract:** Separated populations of wide-ranging ectothermic species may be subjected to differing ambient environmental regimes, with consequences for adult size and related allometric characteristics. We investigated effects of environmentally induced size variation in freshwater Australian Chironomidae (Diptera) on several morphometric ratios used to account for such variation in taxonomic classification. *Echinocladius martini* Cranston, 2000 (Orthoclaadiinae) and *Australopelopia prionopectera* Cranston, 2000 (Tanypodinae) larvae were sampled from tropical and temperate populations separated by approximately 1800 km, and reared to adult at temperatures representing the continent-wide range encountered by these species. Additionally, adults were sampled regularly from a field population to assess seasonal variation. Body size was related inversely to rearing temperature for both species. Nonlinear (allometric) relationships were observed consistently between body size and ratios pertaining to the antennae (AR, the ratio of the terminal to subapical flagellomeres) and legs (BV, the ratio of proximal segments to distal tarsomeres), demonstrating that these ratios inadequately factor out within-species, size-related divergence. Responses of other ratios varied erratically with sex, species, and population. We advocate cautious use of these ratios in taxonomy and phylogenetic reconstruction. We discuss possible adaptive significance of allometry in the male antennae related to the “tuning” of sound-sensitive structures to the expected terrestrial environment encountered at emergence.

**Résumé :** Les populations séparées d'espèces ectothermes à grande répartition géographique peuvent être soumises à des régimes environnementaux ambiants différents qui se reflètent dans la taille des adultes et dans les caractéristiques allométriques associées. Nous avons étudié les effets de la variation de taille provoquée par l'environnement chez des Chironomidae (Diptera) australiens sur divers rapports morphométriques utilisés pour décrire cette variation dans la classification taxonomique. Nous avons prélevé des larves d'*Echinocladius martini* Cranston, 2000 (Orthoclaadiinae) et d'*Australopelopia prionopectera* Cranston, 2000 (Tanypodinae) de populations tropicales et tempérées distantes d'environ 1800 km; nous les avons élevés jusqu'au stade adulte à des températures qui représentent la gamme des températures subies par ces espèces à l'échelle du continent. Nous avons aussi récolté des adultes en nature à intervalles réguliers afin d'évaluer la variation saisonnière. Il y a une relation inverse entre la taille corporelle et la température d'élevage chez les deux espèces. Il y a aussi, de façon constante, des relations non linéaires (allométriques) entre la taille du corps et les rapports relatifs aux antennes (AR, rapport du flagellomère terminal sur les flagellomères subapicaux) et aux pattes (BV, rapport des segments proximaux sur les tarsomères distaux), ce qui démontre que ces rapports n'éliminent pas de façon adéquate la divergence reliée à la taille au sein d'une espèce. Les réponses des autres rapports varient de façon erratique en fonction du sexe, de l'espèce et de la population. Nous suggérons donc d'utiliser avec précaution ces rapports en taxonomie et en reconstruction phylogénétique. La signification adaptative possible de l'allométrie de l'antenne du mâle en relation avec l'ajustement des structures audiosensorielles à l'environnement terrestre escompté à l'émergence fait l'objet d'une discussion.

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## Introduction

In the taxonomy of ectotherms, morphological ratios between body parts have been used implicitly (e.g., by Sæther

1975) to factor out the effects of environmentally induced variation in body size and related characteristics arising from the variable conditions to which widely distributed and ecologically flexible species are exposed. It is assumed that

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**B.G. McKie.**<sup>1</sup> James Cook University, Townsville, Queensland, Australia, and Department of Entomology, University of California, Davis, CA 95616, USA.

**P.S. Cranston.**<sup>2</sup> Department of Entomology, University of California, Davis, CA 95616, USA.

<sup>1</sup>Present address: Department of Ecology and Environmental Science, Umeå University, SE-90187 Umeå, Sweden.

<sup>2</sup>Corresponding author (e-mail: [pscranston@ucdavis.edu](mailto:pscranston@ucdavis.edu)).

even if the overall size of different body parts changes as a function of local environmental conditions, the relative proportions (ratio) of the body parts, being under stabilizing selection for the optimization of function, should remain constant. However, some ratios conceivably may alter in response to changing environmental conditions in order that biological function be maintained, as seen in plants that alter the ratio of roots to shoots in response to local soil conditions (Paz 2003). Many biologically significant functions vary with body size, usually in nonlinear, allometric fashion (Peters 1983). If putatively "taxonomically diagnostic" ratios themselves prove to vary strongly with body size, then not only is their utility in species distinction undermined, but environment-driven phenotypic plasticity of potential biological significance also is implied.

Chironomid midges (Diptera: Chironomidae) are speciose, ecologically significant macroinvertebrates both in their aquatic immature stages and as aerial flying adults. True diversity may be obscured by suspected (e.g., Rosenberg 1971) nonlinear scaling relationships of their body parts, involving inter alia antennae, legs, and wings of adult midges, and any or all "taxonomically diagnostic" ratios derived from these measurements. Such traditional ratios, although recognized to be variable by Edwards (1929, 1931), have become almost de rigueur in descriptive chironomid taxonomy. Suspicions of excessive or uninterpreted variation have been raised (e.g., Prat 1985; Cranston and Oliver 1988; Kobayashi and Hayashi 2001). However, segregation of apparent outlying individuals based primarily on ratios continues to provide justification for erection of new species. This trend, as critiqued by Cranston and Oliver (1988), lacks any statistical framework. As such, purportedly discriminatory ratios need examination under controlled conditions for assessment of correlation with environmentally induced size variation. Significantly, some traditional ratios are likely to reflect biological functions that are subject to local environmental influence. For example, chironomid antennae are involved in sound reception of wing-beat frequencies of conspecifics (Römer and Rosin 1969; Römer 1971), which in turn correlate with ambient temperature (Ogawa 1992). Accordingly, ratios associated with the antennae may change with local thermal conditions to maintain optimal reception of wing-beat frequencies.

Previous investigations of chironomid morphological variation, though often using large samples (e.g., Rosenberg 1971), have focussed on geographically restricted locations and may have under-sampled total morphological variation. Purportedly diagnostic variation in colour pattern has been related experimentally to season and temperature of larval development (e.g., Matena 1995; Kobayashi and Hayashi 2001), and long-term sampling at single sites from natural populations has shown that temperature-dependent colour polymorphism is associated also with adult size variation (Kobayashi and Hayashi 2001). Seasonal colour polymorphism in *Polypedilum* (*Pentapedilum*) *nubens* (Edwards, 1929) (as *Pentapedilum*) was associated also with antennal and dorsocentral setal allometry by Vilchez Quero and Casa Jimenez (1987). Size-dependent changes in morphology of what have been considered otherwise to be highly reliable indicators of species identity, the male hypopygial structures, also has been recognized (Kobayashi 1998).

We investigated the effects of environmentally induced size variation in two freshwater Australian Chironomidae species, *Echinocladius martini* Cranston, 2000 (Orthoclaudiinae) and *Australopelopia prionopectera* Cranston, 2000 (Tanypodinae), on several traditional taxonomic ratios. Ranges of these widespread chironomids extend from subalpine and temperate streams in eastern Australia (including Tasmania) to upland streams in tropical north Queensland, encompassing a range of  $>25^\circ$  in latitude and exposure to aquatic environmental temperatures ranging from  $<7$  to  $>22$  °C (McKie et al. 2005). In formal descriptions of these species, Cranston (2000) recognized extensive morphological variation, but did not address this explicitly. We reared larvae collected from tropical and temperate populations to adult, at temperatures representing those encountered by these species across the continent. We assessed whether experimentally induced morphometric variation occurred in a natural population of tropical *E. martini* adults, sampled using light traps. In the tropics this species has aperiodic development, emerging throughout year, allowing an assessment of seasonal variation in its morphology.

## Materials and methods

### Laboratory experiments: reared adults

Live larvae of *E. martini* and *A. prionopectera* were collected from widely separated (approximately 1800 km apart) tropical and temperate Australian populations. The tropical population was sampled from Birthday and Camp creeks ( $18^\circ 59'S$ ,  $146^\circ 10'E$ ; 800–850 m above sea level (a.s.l.)) in the Paluma range, 80 km north of Townsville, northern Queensland. Both streams flow through closed-canopy tropical rainforest and are subject to a winter low flow – summer high flow seasonality, with an annual water temperature range of 15–22 °C. The temperate population was sampled from Lees, Blundells, and Uriarra creeks, which flow through the Brindabella mountain range ( $35^\circ 22'S$ ,  $148^\circ 49'E$ ; 900 m a.s.l.) to the west of Canberra, Australian Capital Territory. These streams were shaded fully by wet sclerophyll forests dominated by hardwood eucalypts, and have an average annual stream temperature range of 7–13 °C. Winters are cold and wet, with occasional snowfalls; summers are hot and somewhat drier, though monthly rainfall variation is small. Collection protocol is detailed in McKie et al. (2004). After collection, individual larvae were transferred to separate jars and reared to adult emergence within controlled environment chambers. Tropical-population animals were reared at 12, 18, 26, or 32 °C, whereas temperate-population individuals were reared at the first three temperatures only, as it proved difficult to collect large numbers of these animals. Initial replication for *E. martini* was in excess of 30 larvae per treatment for the tropical population, but only 20 for the temperate populations. For the predacious *A. prionopectera*, initial replication was lower (10–15 larvae per treatment). During day 1 of the experiment, temperatures gradually were adjusted to treatment levels from 18 °C for the tropical animals and 12 °C for the temperate animals (i.e., from temperatures these populations typically experience). Light was provided from above, on a 12 h light : 12 h dark cycle. Local diel cycles (B.G. McKie, personal observa-

tion) were approximated by reducing temperatures by 2–2.5 °C at night.

Each rearing jar contained 100 mL of water from appropriate collection sites (Birthday Creek for tropical animals, Blundell's Creek for temperate animals), and five drops of a detrital suspension, prepared by filtering particulate material from the same collection sites through a 63- $\mu$ m sieve to exclude other macroinvertebrates. This material was used by *E. martini* larvae for food and in tube construction, and was supplemented as necessary over the experimental period. *Australopelopia prionopectera* larvae were fed every 3rd day with chopped *Lumbriculus variegatus* (Müller, 1774) (Oligochaeta: Lumbriculidae), sold commonly as aquarium fish food ("blackworms"). Larvae used mechanoreception to detect chopped blackworm fragments, which remained active while regenerating into new individuals.

Emergent adults, killed after allowing 24 h for the cuticle to harden, were dissected and slide mounted, according to the protocol detailed in McKie et al. (2004).

### Wild-caught adults: light-trap samples

Adult chironomids were sampled from the tropical population at regular (mostly monthly) intervals from December 1992 to October 1994, with an additional sample collected in June 1999. A single light trap was placed adjacent to Birthday Creek at dusk for one night on each sampling occasion. Insects were attracted by a white fluorescent light, and stunned by fumes rising from ethanol kept in a container below the light. Stunned insects fell into the alcohol, in which they died and were preserved for later dissection. Few *A. prionopectera* or female *E. martini* were attracted, thus results are presented for male *E. martini* only.

### Measurements: the evaluated ratios

For both laboratory-reared and wild-caught adults, the length of the wings, from arculus to wingtip, and wing-vein-, leg-, and antennal-segment lengths were measured at high magnification (40 $\times$ –200 $\times$ ) with the aid of a graticule. Wing length, which correlates with body mass in chironomids (e.g., McLachlan 1986; Xue and Ali 1994), was used as a measure of size in regression analyses (see below).

Wing-vein-, foreleg-, and antennal-segment lengths were used to calculate the following ratios, which are commonly used in chironomid taxonomy:

- AR, antennal ratio, defined originally by Edwards as "Length of last segment of male antenna in relation to the remaining segments of the flagellum ... expressed as a decimal and designated by the letters A.R. (antennal ratio)" (Edwards 1929, p. 283). In current terminology this equates to the ratio of the length of ultimate flagellomere to the combined lengths of flagellomeres 1 to penultimate. A modification has been made for the subfamily Tanypodinae, in which it is the ratio of the length of (the short) ultimate and penultimate flagellomeres to the combined lengths of flagellomeres 1 to pre-penultimate. For the females of all subfamilies the AR is the ratio of the length of the terminal to the combined subterminal flagellomeres.
- LR, leg ratio, which is the ratio of tarsomere<sub>1</sub> length to tibia length. This ratio also owes its definition to Edwards who, as with AR, stated that "the figure ... is usually an approximate estimate rather than an exact measurement,

since experience has shown that there is considerable variation" (Edwards 1929, p. 283).

- BV, "Beinverhältnisse", is the ratio of the combined lengths of femur plus tibia plus tarsomere 1 to the combined lengths of tarsomeres 2–5. This ratio owes its definition to Pagast (1947) who introduced the ratio in a brief "Verzeichnis der Abkürzungen", with a modest elaboration (but an incorrectly transcribed formula) a few pages later.
- SV, "Schenkel-Schine-Verhältnisse", is the ratio of the combined lengths of femur plus tibia to the length of tarsomere 1. This ratio owes its definition also to Pagast (1947) in the same short note as his BV ratio, but with the formula correctly transcribed a few pages later.
- VR, venarum ratio, is the ratio of length of wing vein Cu<sub>1</sub> to the length of vein M, and has been recognized as informative by Fittkau (1962). Hereinafter referred to as wing ratio.

### Analysis

Effects of temperature on overall organism size were assessed using MANOVA, with all 12 size measures (wing length, 2 wing-vein-length measures, 2 antennal measures, and 7 foreleg measures) fitted as dependent variables and temperature and sex fitted as factors. Measures were natural log transformed where necessary to satisfy parametric assumptions. Pillai's trace was used as the test statistic, because of its robustness to small sample sizes, but tests using Roy's largest root (which is useful when autocorrelation among the dependent variables is high; see Hand and Taylor 1987) yielded similar results. Because of uneven distribution of midges between the sexes, the temperature  $\times$  sex interaction could not be tested meaningfully in some analyses, and so was not fitted.

Relationships between size and each of the five ratios were investigated for each species using regression analysis. Analyses were separated by sex, since ratios differed markedly between males and females. For each ratio, three regressions against wing length were carried out, the first combining data for both populations; and two fitting data for the northern and southern populations, respectively. Further regressions were carried out against an alternative size measure, differing according to the ratio involved: AR data were regressed against the length of the terminal antennal segment (or terminal + penultimate for *A. prionopectera*), VR data were regressed against Cu-vein length, and the three foreleg-segment ratios (LR, BV, SV) were regressed against foretarsal length.

MANOVA was used to assess the effect of temperature on overall "organism morphology", with all five measured ratios fitted as dependent variables. Following this analysis, separate ANOVAs were conducted to assess the effect of temperature on each trait in isolation. MANOVA helps protect against type I error inflation caused by conducting such multiple separate tests, but as a further precaution, Bonferroni's correction was applied to the ANOVA results, reducing the effective  $\alpha$  value to 0.01. Separate ANOVAs were conducted for males and females.

Regression analyses for wild-caught *E. martini* males matched those for the laboratory-reared adults. Differences

**Table 1.** Temperature effects on adult midge size in laboratory experiments.

Species and population	Factor	Pillai's trace	<i>F</i>	df	<i>p</i>
<i>Echinocladius martini</i>					
Tropical	Temperature	1.11	5.43	24,104	<0.001
	Sex	1.00	1412.60	12,51	<0.001
	Temperature × sex	0.56	1.67	24,104	0.040
Temperate	Temperature	1.28	2.66	24,36	0.004
	Sex	1.00	1025.10	12,17	<0.001
	Temperature × sex	0.85	1.10	24,36	0.390
<i>Australopelopia prionopectera</i>					
Tropical	Temperature	1.61	3.05	24,18	0.009
	Sex	1.00	300.96	12,8	<0.001
Temperate	Temperature	1.68	3.07	24,14	0.016
	Sex	1.00	1335.02	12,6	<0.001

**Note:** Output from MANOVA with temperature and sex fitted as factors and 12 size measures fitted as response variables. Two-way interaction terms were not fitted for *A. prionopectera*, because the distribution of the sexes among temperatures was too uneven to analyse.

in ratios according to sample date were tested for using MANOVA and ANOVA.

In all cases, only individuals from which robust measures of the relevant trait were recorded were included in analyses. SPSS® for Windows version 10.0.5 (SPSS Inc. 1989–1999) was used for analyses.

## Results

### Laboratory-reared adults

Temperature affected overall body size significantly for both populations of both species (Table 1). Typical is the effect of temperature on wing length: animals reared at warmer temperatures emerge with smaller wings (Fig. 1). Southern population individuals consistently had longer wings at all temperatures for both species (Fig. 1).

Full output from regressions of the ratios against wing length and the alternative size measures are given in Tables 2 and 3, respectively. Since antennal (AR) and leg (LR) ratios are traditional and universal in chironomid species descriptions, we focus on these results.

The AR of male *E. martini* increased with increasing wing length, with a high  $r^2$  value (85%) indicating a robust fit of the data to the model (Fig. 2a). This relationship was apparent in both combined and separate population analyses. Regression of male AR data against antennal terminal segment length yielded similar results, with  $r^2$  values a little higher (Fig. 2b).

In contrast, female AR decreased with increasing wing length (Fig. 2c). This relationship was apparent only when data for both populations was combined, and the fit of the data to the model was weak ( $r^2 = 14\%$ ). When fitted against the alternative size measure (Fig. 2d), these results were reversed: there was no relationship in the combined populations analysis, but there were significant positive relationships between AR and antennal terminal segment length when data for the tropical and temperate populations were analysed separately, with  $r^2$  values of 21% and 51%, respectively.

The relationship between male *A. prionopectera* AR and wing length was not significant ( $p = 0.06$ ) for the combined-population analysis, but significant for the southern-population analysis (Fig. 3a). When fitted against terminal

antennal segment length, the relationship was significant for both northern- and southern-population data in isolation and for the combined data set (Fig. 3b). In all cases, the relationships were positive, but weak (all  $r^2 < 20\%$ ).

Female *A. prionopectera* AR was related negatively to wing length but positively to terminal antennal segment length in the combined-population analysis (Fig. 3c–3d). This seems to be driven by divergence in the size of the terminal antennal segments with temperate-population females having shorter terminal antennal segments ( $146.9 \pm 1.1 \mu\text{m}$  (mean  $\pm$  SE),  $n = 11$ ) than those from the tropics ( $169.5 \pm 1.2 \mu\text{m}$ ,  $n = 8$ ). In the separate-population analyses, AR for both northern and southern populations was related positively to terminal antennal segment length, but not wing length (Fig. 3c–3d). Model fit was good in significant regressions ( $r^2 > 60\%$ ), with  $r^2$  values greater than those for corresponding female *E. martini* and male *A. prionopectera* analyses, though lower than for male *E. martini*.

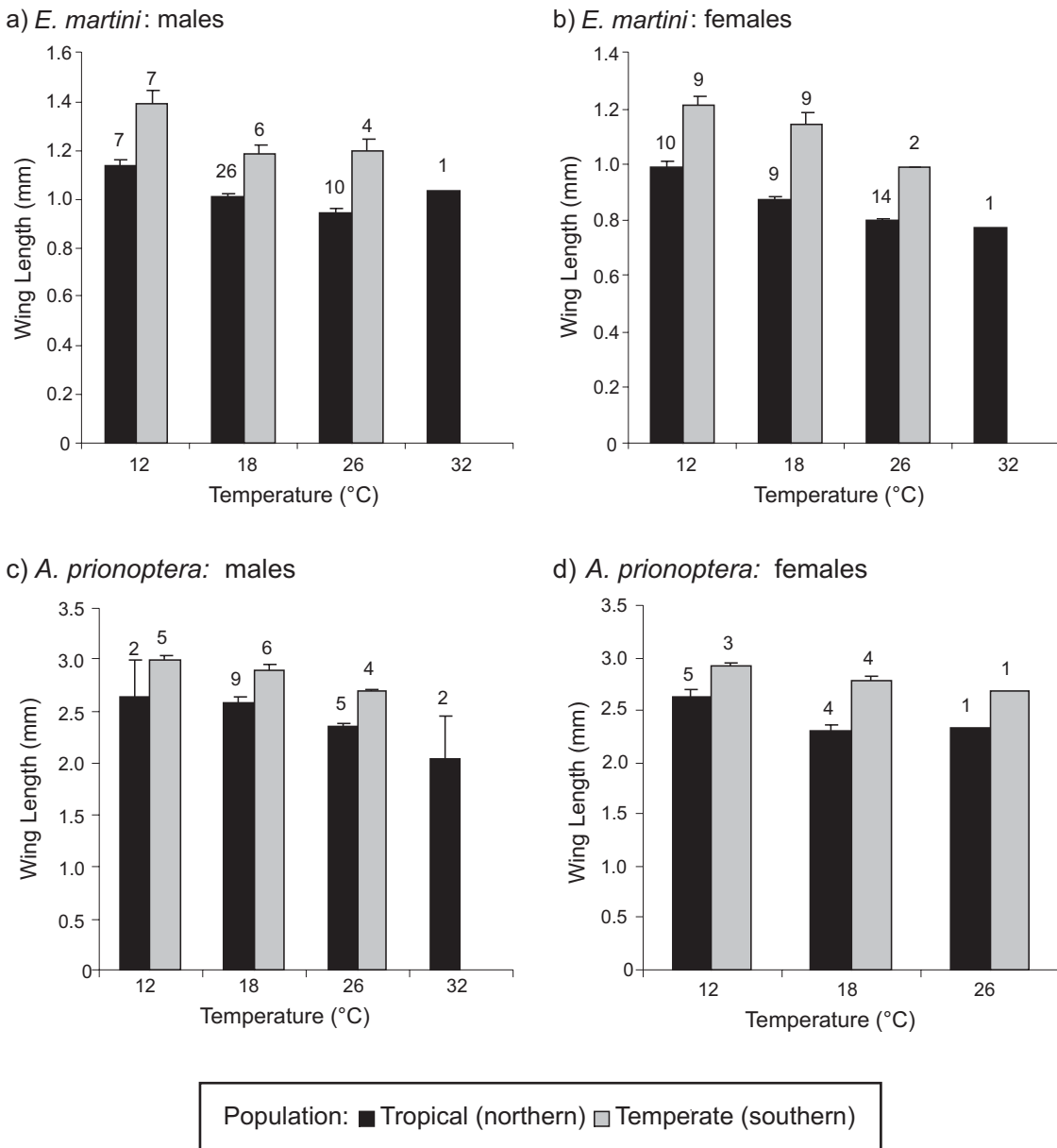
The LR of *E. martini* males was related negatively to both wing length and foretibial length, but only in the combined-population analyses (Fig. 4a–4b), with weak model fits ( $r^2 < 20\%$ ). Female LR was related positively to wing length and foretibial length for the combined-population analyses (Fig. 4c–4d). In the separate-population analyses, LR was related positively to foretibial length for the southern population only (Fig. 4d). In both cases,  $r^2$  values were low ( $<25\%$ ).

*Australopelopia prionopectera* LR was not related significantly to either wing or foretibial lengths for either males or females, regardless of whether the analysis was for the separate populations or the combined data set (Fig. 5a–5d).

Data for the remaining ratios is not plotted. The BV ratio was related negatively to both wing and foretibial lengths for both male and female *E. martini* when data for both populations were combined, with moderate  $r^2$  values (30%–50%); similar relationships were inconsistent when the two populations were analysed separately (Tables 2, 3). For *A. prionopectera*, the BV ratio was related significantly to wing length for males in the combined-data analysis only (Table 2). In contrast with *E. martini*, the relationship was positive, but the  $r^2$  value was small (15%).

In the combined-population analyses, the SV ratio was related positively to wing and foretibial lengths for *E. martini*

**Fig. 1.** Laboratory experiments: effect of temperature on wing lengths of (a, b) *Echinocladius martini* males and females and (c, d) *Australopelopia prionopectera* males and females. The bars are means  $\pm$  SE, with the number of observations indicated above each bar. ANOVA results (sexes pooled) — tropical *E. martini*:  $F_{[2,69]} = 59.14, p < 0.001^*$ ; temperate *E. martini*:  $F_{[2,31]} = 7.26, p = 0.003$ ; tropical *A. prionopectera*:  $F_{[2,22]} = 4.60, p = 0.021^*$ ; temperate *A. prionopectera*:  $F_{[2,19]} = 9.04, p = 0.002$ . \*The 32 °C treatment was excluded from the ANOVA because of insufficient replicates. See Table 1 for the global MANOVA test results. Modified from McKie et al. (2004).



males but negatively for females, with low  $r^2$  values (Tables 2, 3). Similar results were apparent in most separate-population analyses for females but not males. For *A. prionopectera*, there were no significant relationships between SV ratio and wing length for either sex (Table 2).

The wing ratio (VR) was related positively to wing and Cu-vein lengths for male but not female *E. martini* when data for both populations were combined (Tables 2, 3), though the result was near significance for females when fitted against Cu-vein length ( $p = 0.056$ , Table 3). These relationships also were significant or marginally significant ( $p = 0.082$ ) in the separate-population analyses for males (Tables 2, 3). In all cases,  $r^2$  values were low, though generally higher when fitted against the Cu-vein length. Wing ratio

was related positively to wing and Cu-vein lengths (Tables 2, 3) for both male and female *A. prionopectera* when data for both populations were combined, with  $r^2$  values low to moderate (29%–50%). These relationships were significant in only one separate-population analysis (tropical males fitted against Cu-vein length; Table 3) and marginally significant ( $p = 0.053$ ) in another (southern *A. prionopectera* fitted against wing length; Table 2).

MANOVA analysis indicates temperature affected overall “organism morphology”, as measured by the five ratios for both northern and southern *E. martini* (Table 4). In separate analyses of data for the five ratios, temperature significantly affected male AR and wing ratio and female SV ratio for northern *E. martini* (Fig. 6a–6c). Female BV ratio also was

**Table 2.** Relationships between wing length (body size) and ratios in laboratory experiments.

Species and sex	Ratio*	Both populations combined					Tropical population only		
		<i>n</i>	Slope	<i>r</i> <sup>2</sup>	<i>F</i>	<i>p</i>	<i>n</i>	Slope	<i>r</i> <sup>2</sup>
<i>Echinocladius martini</i>									
Males	Antennal	56	$4.15 \times 10^{-4}$	0.85	309.28	<0.001	40	$3.49 \times 10^{-4}$	0.61
	Leg	55	$-2.96 \times 10^{-5}$	0.08	4.69	0.035	39	$-4.06 \times 10^{-6}$	0.01
	BV	55	$-2.41 \times 10^{-4}$	0.33	26.48	<0.001	39	$-1.52 \times 10^{-4}$	0.06
	SV	55	$2.14 \times 10^{-4}$	0.15	9.45	0.003	39	$5.64 \times 10^{-5}$	0.00
	Wing	55	$1.33 \times 10^{-4}$	0.185	12.01	0.001	39	$2.17 \times 10^{-4}$	0.15
Females	Antennal	52	$-1.18 \times 10^{-4}$	0.14	8.42	0.006	33	$-1.85 \times 10^{-5}$	0.00
	Leg	51	$2.93 \times 10^{-5}$	0.12	6.51	0.014	32	$3.50 \times 10^{-5}$	0.04
	BV	51	$-5.17 \times 10^{-4}$	0.46	41.74	<0.001	32	$-4.17 \times 10^{-4}$	0.12
	SV	51	$-2.24 \times 10^{-4}$	0.19	11.51	0.001	32	$-4.23 \times 10^{-4}$	0.21
	Wing	49	$5.19 \times 10^{-5}$	0.051	2.52	0.119	31	$6.34 \times 10^{-5}$	0.03
<i>Australopelopia prionopectera</i>									
Males	Antennal	26	$9.93 \times 10^{-5}$	0.138	3.84	0.062	13	$8.86 \times 10^{-5}$	0.08
	Leg	28	$-1.01 \times 10^{-6}$	0.00	0.00	0.963	14	$1.21 \times 10^{-5}$	0.01
	BV	28	$1.47 \times 10^{-4}$	0.15	4.44	0.045	14	$-3.01 \times 10^{-4}$	0.12
	SV	28	$-1.30 \times 10^{-4}$	0.05	1.41	0.246	14	$7.50 \times 10^{-6}$	0.00
	Wing	28	$4.64 \times 10^{-5}$	0.43	19.96	<0.001	13	$4.04 \times 10^{-5}$	0.21
Females	Antennal	18	$-1.42 \times 10^{-4}$	0.64	28.37	<0.001	10	$-1.35 \times 10^{-4}$	0.43
	Leg	18	$-1.26 \times 10^{-5}$	0.02	0.35	0.565	10	$-2.71 \times 10^{-5}$	0.04
	BV	18	$7.52 \times 10^{-5}$	0.08	1.46	0.244	10	$2.91 \times 10^{-5}$	0.00
	SV	18	$7.81 \times 10^{-5}$	0.06	0.93	0.350	10	$-1.54 \times 10^{-5}$	0.00
	Wing	18	$4.72 \times 10^{-5}$	0.29	8.07	0.012	10	$6.05 \times 10^{-6}$	0.01

**Note:** Output from regression analyses for combined population data and for separate populations.

\*Antennal ratio is abbreviated to AR in the text; leg ratio is abbreviated to LR in the text; BV is the ratio of proximal segments to distal tarsomeres; and SV is the ratio of the combined lengths of femur plus tibia to the length of tarsomere 1.

**Table 3.** Relationships between alternative size measures and ratios in laboratory experiments.

Species and sex	Ratio*	Both populations combined					Tropical population only		
		<i>n</i>	Slope	<i>r</i> <sup>2</sup>	<i>F</i>	<i>p</i>	<i>n</i>	Slope	<i>r</i> <sup>2</sup>
<i>Echinocladius martini</i>									
Males	Antennal	56	$1.48 \times 10^{-3}$	0.90	491.90	<0.001	40	$1.40 \times 10^{-3}$	0.69
	Leg	55	$-8.98 \times 10^{-5}$	0.08	4.49	0.039	39	$-9.00 \times 10^{-6}$	0.00
	BV	55	$-6.94 \times 10^{-4}$	0.29	21.30	<0.001	39	$-2.87 \times 10^{-4}$	0.03
	SV	55	$6.90 \times 10^{-4}$	0.16	10.37	0.002	39	$2.81 \times 10^{-4}$	0.02
	Wing	55	$3.58 \times 10^{-4}$	0.27	19.83	<0.001	39	$5.58 \times 10^{-4}$	0.27
Females	Antennal	52	$9.82 \times 10^{-4}$	0.01	0.71	0.405	33	$4.58 \times 10^{-3}$	0.21
	Leg	51	$1.01 \times 10^{-4}$	0.08	4.09	0.049	32	$8.17 \times 10^{-6}$	0.00
	BV	51	$-1.95 \times 10^{-3}$	0.36	27.66	<0.001	32	$-8.44 \times 10^{-4}$	0.03
	SV	51	$-6.86 \times 10^{-4}$	0.10	5.33	0.025	32	$-7.64 \times 10^{-4}$	0.04
	Wing	49	$1.65 \times 10^{-4}$	0.08	3.83	0.056	31	$1.77 \times 10^{-4}$	0.04
<i>Australopelopia prionopectera</i>									
Males	Antennal	26	$6.84 \times 10^{-4}$	0.42	17.50	<0.001	13	$7.07 \times 10^{-4}$	0.38
	Leg	28	$-4.60 \times 10^{-5}$	0.07	1.92	0.178	14	$-2.06 \times 10^{-5}$	0.01
	BV	28	$2.16 \times 10^{-4}$	0.12	3.61	0.068	14	$1.35 \times 10^{-4}$	0.06
	SV	28	$-1.46 \times 10^{-4}$	0.03	0.67	0.420	14	$-3.40 \times 10^{-4}$	0.12
	Wing	28	$1.88 \times 10^{-4}$	0.50	25.58	<0.001	13	$1.73 \times 10^{-4}$	0.40
Females	Antennal	18	$2.22 \times 10^{-3}$	0.75	48.98	<0.001	10	$2.32 \times 10^{-3}$	0.61
	Leg	18	$-5.98 \times 10^{-5}$	0.10	1.76	0.203	10	$-5.12 \times 10^{-5}$	0.30
	BV	18	$-5.95 \times 10^{-5}$	0.01	0.18	0.679	10	$-7.11 \times 10^{-5}$	0.18
	SV	18	$7.48 \times 10^{-5}$	0.01	0.17	0.686	10	$3.14 \times 10^{-5}$	0.00
	Wing	18	$1.82 \times 10^{-4}$	0.35	8.70	0.009	10	$3.81 \times 10^{-5}$	0.04

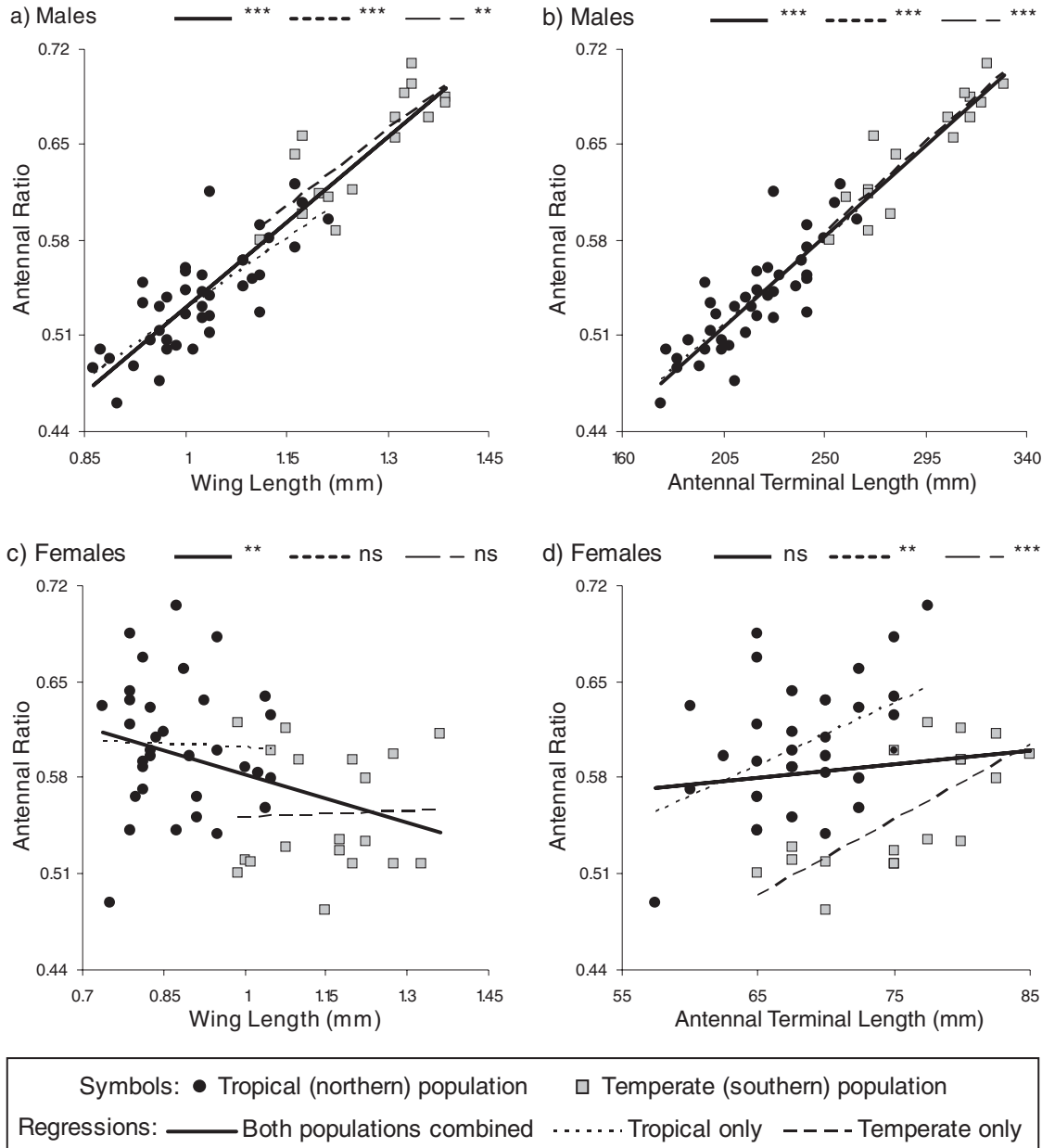
**Note:** Output from regression analyses for combined population data and for separate populations.

\*Ratios based on alternative size measures are as follows: antennal ratio (AR in the text) fitted against antennal terminal segment length; leg (LR in the text), BV, and SV ratios fitted against foretibial length; wing ratio fitted against Cu-vein length.

Southern population only						
<i>F</i>	<i>p</i>	<i>n</i>	Slope	<i>r</i> <sup>2</sup>	<i>F</i>	<i>p</i>
60.16	<0.001	16	$3.75 \times 10^{-4}$	0.65	26.46	0.001
0.17	0.687	16	$-1.04 \times 10^{-6}$	0.00	0.64	0.436
0.17	0.118	16	$-3.77 \times 10^{-4}$	0.32	6.64	0.022
0.17	0.465	16	$1.81 \times 10^{-4}$	0.04	0.84	0.374
6.55	0.015	16	$1.13 \times 10^{-4}$	0.20	3.51	0.082
0.04	0.841	19	$1.21 \times 10^{-5}$	0.00	0.02	0.897
1.35	0.253	19	$6.58 \times 10^{-5}$	0.23	4.98	0.039
4.03	0.054	19	$-5.60 \times 10^{-4}$	0.48	15.75	0.001
8.19	0.008	19	$-4.80 \times 10^{-4}$	0.42	12.25	0.002
0.88	0.357	18	$3.43 \times 10^{-5}$	0.01	0.15	0.708
0.94	0.208	13	$1.47 \times 10^{-4}$	0.14	1.79	0.038
0.08	0.786	14	$5.69 \times 10^{-6}$	0.00	0.02	0.897
1.66	0.222	14	$-2.06 \times 10^{-4}$	0.09	1.26	0.284
0.00	0.959	14	$5.83 \times 10^{-5}$	0.02	0.25	0.628
2.87	0.120	15	$2.66 \times 10^{-5}$	0.26	4.52	0.053
6.15	0.353	8	$-2.43 \times 10^{-5}$	0.07	0.43	0.538
0.36	0.566	8	$-4.17 \times 10^{-5}$	0.14	1.00	0.356
0.03	0.878	8	$1.61 \times 10^{-4}$	0.35	3.27	0.120
0.03	0.876	8	$-1.71 \times 10^{-4}$	0.14	0.95	0.368
0.09	0.767	8	$-3.35 \times 10^{-5}$	0.11	0.75	0.421

Southern population only						
<i>F</i>	<i>p</i>	<i>n</i>	Slope	<i>r</i> <sup>2</sup>	<i>F</i>	<i>p</i>
85.81	<0.001	16	$1.46 \times 10^{-3}$	0.83	67.02	<0.001
0.01	0.908	16	$-3.52 \times 10^{-5}$	0.00	0.05	0.829
1.16	0.289	16	$-1.33 \times 10^{-3}$	0.33	6.95	0.020
0.55	0.465	16	$7.15 \times 10^{-4}$	0.06	0.84	0.374
13.76	0.001	16	$2.72 \times 10^{-4}$	0.25	4.68	0.048
8.28	0.007	19	$5.52 \times 10^{-3}$	0.51	17.44	<0.001
0.00	0.951	19	$2.19 \times 10^{-4}$	0.18	3.58	0.075
0.82	0.373	19	$-1.82 \times 10^{-3}$	0.35	9.09	0.008
1.20	0.283	19	$-1.38 \times 10^{-3}$	0.24	5.28	0.034
1.17	0.288	18	$1.91 \times 10^{-4}$	0.05	0.90	0.357
6.81	0.024	13	$8.07 \times 10^{-4}$	0.44	8.79	0.013
0.17	0.686	14	$-8.68 \times 10^{-5}$	0.22	3.31	0.094
0.73	0.409	14	$2.06 \times 10^{-4}$	0.16	2.29	0.156
1.60	0.230	14	$1.59 \times 10^{-4}$	0.04	0.45	0.517
7.41	0.020	15	$9.22 \times 10^{-5}$	0.18	2.86	0.115
12.54	0.008	8	$8.44 \times 10^{-4}$	0.47	5.23	0.062
0.64	0.448	8	$-1.08 \times 10^{-4}$	0.07	2.58	0.159
0.27	0.615	8	$-6.93 \times 10^{-5}$	0.01	0.04	0.844
0.01	0.908	8	$2.75 \times 10^{-4}$	0.32	2.84	0.143
0.31	0.591	8	$4.87 \times 10^{-6}$	0.00	0.00	0.972

**Fig. 2.** Laboratory experiments: *E. martini* antennal ratio plotted against (a, c) wing length and (b, d) antennal terminal segment length. Significance of the values for the regression lines are indicated above the figures (ns,  $p > 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ). See Tables 2 and 3 for the complete regression results.



significant at the 5% level, but not after the Bonferroni correction (Fig. 6d). Male AR and wing ratio declined with increasing temperature (Fig. 6a–6b), whereas BV and SV ratios increased with increasing temperature for females (Fig. 6c–6d).

No ratios were affected significantly by temperature following Bonferroni correction for southern *E. martini* or *A. prionopectera*, but replication was limited (all  $p \gg 0.05$ ).

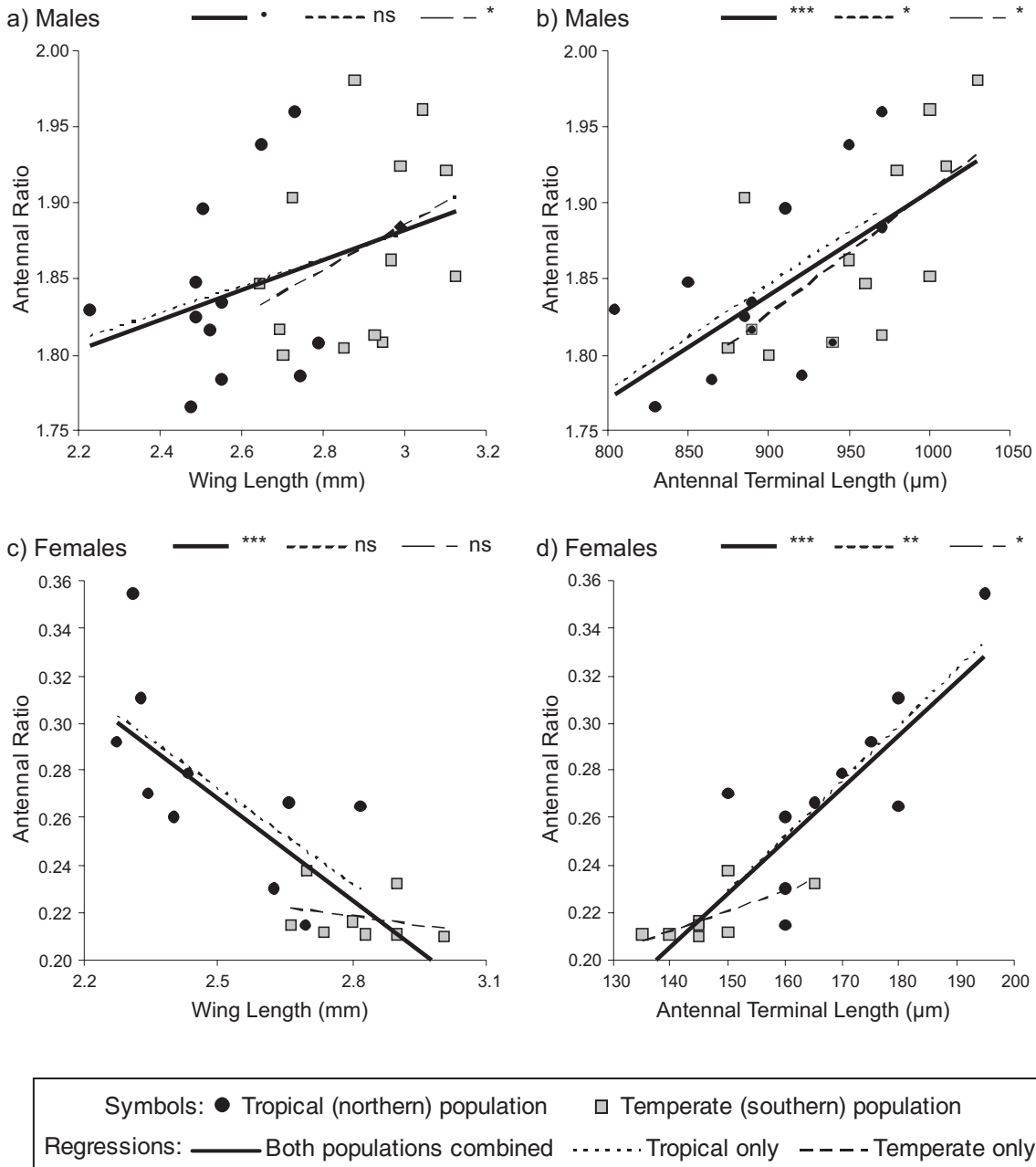
**Wild-caught adults: tropical population**

The antennal ratio of wild-caught male *E. martini* from the tropical population was related strongly to both wing and antennal terminal segment lengths (Table 5, Fig. 7a–7b),

matching results for laboratory-reared adults (above). The BV ratio was related negatively to both foretibial and wing lengths, and a positive relationship existed between wing ratio and Cu-vein length, but not between wing ratio and total wing length (Table 5, data not plotted). The LR and SV ratio were not related significantly to either wing length or foretibial lengths (Table 5, LR data; Fig. 7c–7d, SV data not plotted), in contrast with laboratory results.

Overall morphology, as assessed with all five ratios, differed significantly among samples (MANOVA, sample date: Pillai's trace = 1.304,  $F_{[40,260]} = 2.29$ ,  $p < 0.001$ ). Specifically, the AR and BV ratio varied significantly among samples, with ratios generally larger in cool (April, June,

**Fig. 3.** Laboratory experiments: *A. prionoptera* antennal ratio (AR in the text) plotted against (a, c) wing length and (b, d) antennal terminal segment length. Significance of the values for the regression lines are indicated above the figures (ns,  $p \gg 0.05$ ; ●,  $p < 0.1$  (ns); \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ). See Tables 2 and 3 for the complete regression results.



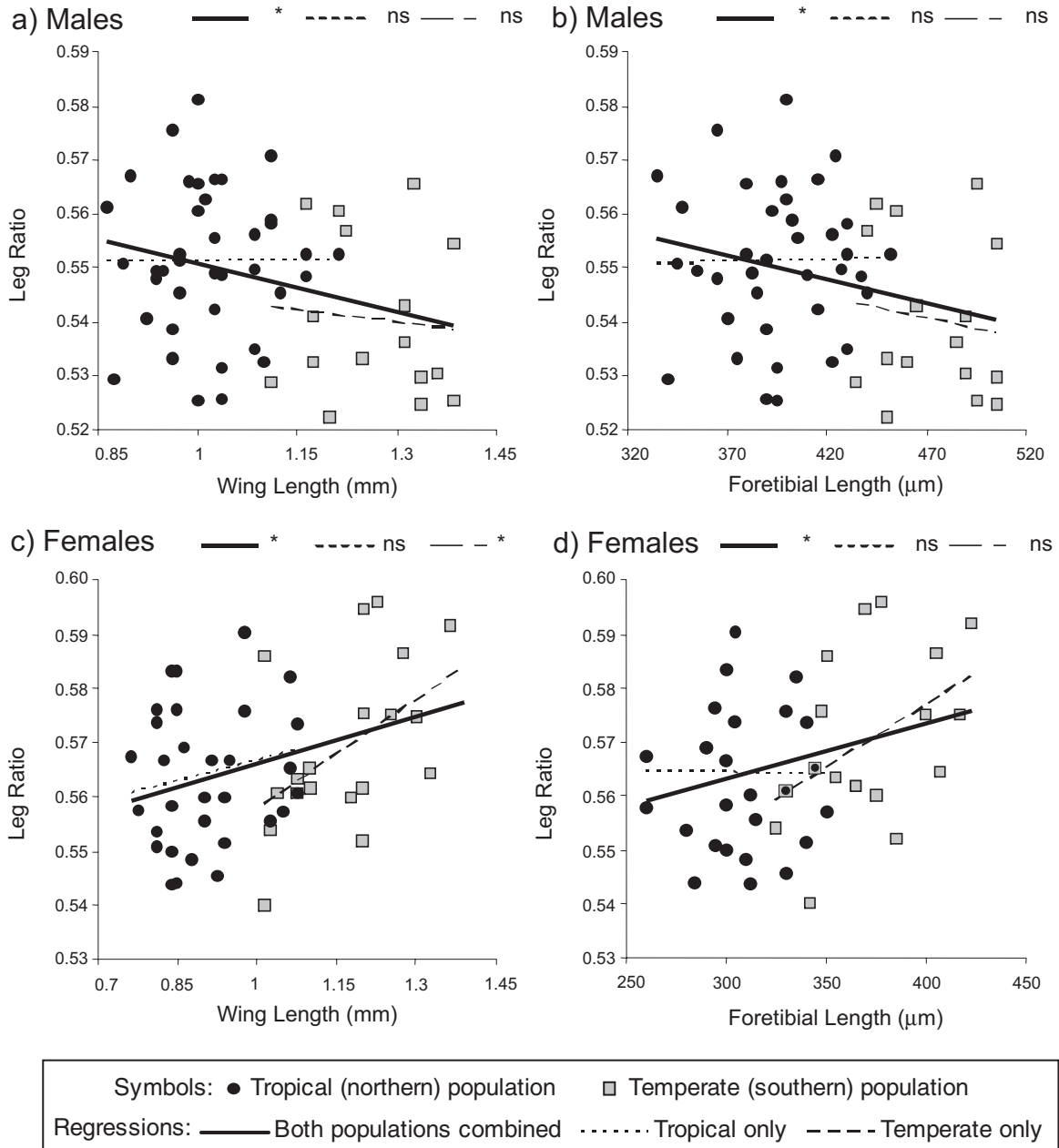
September) months than in warm months (Fig. 8). No similar variation was apparent for the LR, SV ratio, or wing ratio (all  $p \gg 0.05$ ).

**Discussion**

Experimental verification that wing length, a strong correlate of adult body size in chironomids (e.g., McLachlan 1986), was related inversely to the temperature of development of the immature stages was expected, and matched results obtained for aquatic poikilotherms with broad temperature tolerances (reviewed by McKie et al. 2004). Size di-

vergence in adults from tropical and temperate populations may conform to Bergmann’s rule, a generalization based originally on data for terrestrial vertebrates that animal populations from colder climates are larger bodied than those from warmer climates (Partridge and Coyne 1997, discussed in McKie et al. 2004). Though not observed consistently for ectotherms (Mousseau 1997), this relationship has been observed previously for Australian Chironomidae — *Chironomus tentans* Fabricius, 1805 were collected as eggs from a cool temperate site in Victoria (Werribee) and a hot site in Western Australia (Kalgoorlie) and reared to adult at 20 °C (by J. Martin, University of Melbourne), with those from the

**Fig. 4.** Laboratory experiments: *E. martini* leg ratio (LR in the text) plotted against (a, c) wing length and (b, d) foretibial length. Significance of the values for the regression lines are indicated above the figures (ns,  $p > 0.05$ ; \*,  $p < 0.05$ ). See Tables 2 and 3 for the complete regression results.



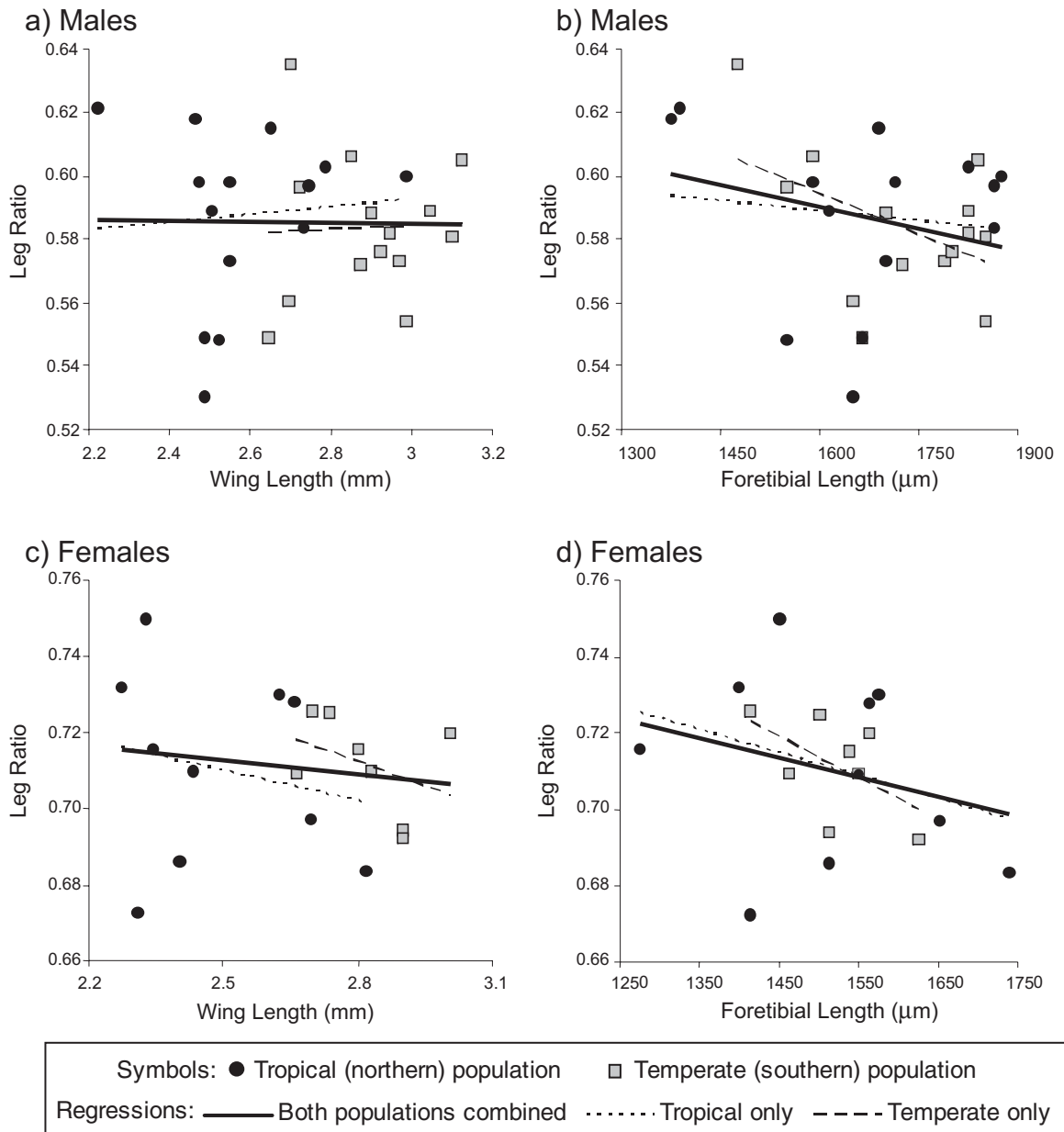
temperate site consistently larger (Victorian population wing length  $3.74 \pm 0.30$  mm,  $n = 25$ ; Western Australian population  $3.09 \pm 0.28$  mm,  $n = 6$ ;  $t = -4.8$ ,  $p < 0.001$ ; B.G. McKie, personal observation). However, the range of variation in some diagnostic ratios both between and within populations, and seasonally within the field population, was unexpected, especially given the typical use of such data in species discrimination and phylogenetic studies. These results have both systematic and ecological implications.

**Systematic implications**

Though stated explicitly neither by the originators of morphometric ratios nor by subsequent users, the intention of converting mensural features to ratios is to correct for

allometry. In this study, all ratios for male *E. martini* related significantly to body size (assessed using wing length), as did all except the wing ratio (VR) for females. For *A. prionoptera*, relationships between wing length and AR and wing ratio were significant or nearly so for both sexes, with the BV ratio additionally significant for males. However,  $r^2$  values generally were low for LR and wing ratio relationships of both species. This reflects in part the limited range of variation typical of the ratios, with some significant only when variation was extended by combining data for southern and northern populations, which coupled with small sample sizes (especially for the difficult to rear *A. prionoptera*), caused signals to remain weak and hard to detect. Because these ratios apparently do obscure the allometric

**Fig. 5.** Laboratory experiments: *A. prionopectera* leg ratio (LR in the text) plotted against (a, c) wing length and (b, d) foretibial length. Regression lines are not significant (all  $p \gg 0.05$ ). See Tables 2 and 3 for complete regression results.



signal (if only by reducing range variation), with caution they may have some place in taxonomic morphometrics. However, for AR, especially in males of *E. martini* and females of *A. prionopectera*, the allometric size relationship was strong and tight, indicative of a ratio exhibiting substantial environmentally driven phenotypic plasticity. This is supported by our detection of the same relationship in the field population, in which AR varied markedly with season, presumably under ambient-temperature control.

Overall, the two most consistently significant ratios (significant in most combined- and separate-population analyses, and for wild-caught *E. martini*) were AR and BV. The BV ratio is the only LR that incorporates information about all leg segments; the LR and SV are partial and the wing ratio lacks information about breadth of the wing and loca-

tions of other veins and cells. Missing information reduces the likelihood of detection of biologically significant allometric plasticity in ratios pertaining to complete “morphological units” (the entire leg or wing). Whether this increases their value in species discrimination depends on relationships with overall morphology.

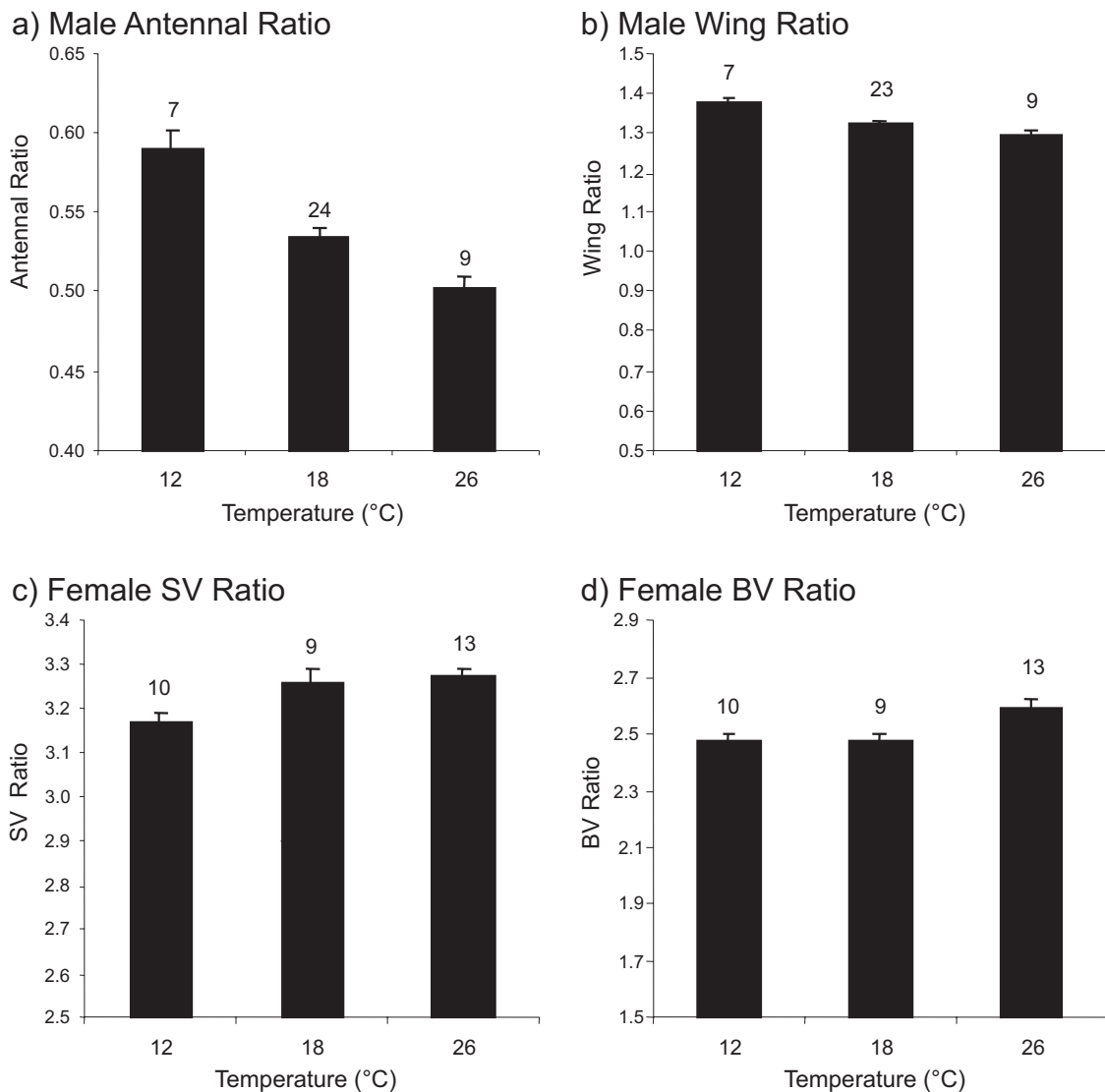
Despite the significance of some ratios, there were several anomalies. For example, the AR of female *A. prionopectera* was inversely proportional to wing length but was positively related to antennal terminal length, and the slopes of the regressions differed between northern and southern populations. In contrast, for female *E. martini*, AR did not vary within populations, but there was divergence between populations when wing length was used as the size measure. When terminal segment length was used as the size measure,

**Table 4.** Temperature effects on organism morphology.

Species and population	Factor	Pillai's trace	F	df	p
<i>Echinocladus martini</i>					
Tropical	Temperature	0.53	4.25	10,118	<0.001
	Sex	0.83	56.67	5,58	<0.001
	Temperature × sex	0.34	2.43	10,118	0.012
Temperate	Temperature	0.60	2.12	10,50	0.040
	Sex	0.69	10.58	5,24	<0.001
	Temperature × sex	0.48	1.56	10,50	0.145
<i>Australopelopia prionopectera</i>					
Tropical	Temperature	0.29	0.55	10,32	0.845
	Sex	1.00	656.76	5,15	<0.001
Temperate	Temperature	0.56	1.09	10,28	0.404
	Sex	1.00	889.41	5,13	<0.001

**Note:** Output from MANOVA with temperature and sex fitted as factors and the five ratios fitted as response variables. Two-way interaction terms were not fitted for *A. prionopectera*, because the distribution of the sexes among temperatures was too uneven to analyse.

**Fig. 6.** Laboratory experiments: effect of temperature on tropical *E. martini* male (a) antennal ratio (AR in the text) and (b) wing ratio, and on *E. martini* female (c) SV and (d) BV ratios. SV is the ratio of the combined lengths of femur plus tibia to the length of tarsomere 1 and BV is the ratio of proximal segments to distal tarsomeres. The bars are means ± SE, with the number of observations indicated above each bar. ANOVA results: (a)  $F_{[2,37]} = 22.62, p < 0.001$ ; (b)  $F_{[2,36]} = 7.34, p = 0.002$ ; (c)  $F_{[2,29]} = 6.34, p = 0.005$ ; (d)  $F_{[2,29]} = 6.34, p = 0.020$  (not significant after Bonferroni correction).



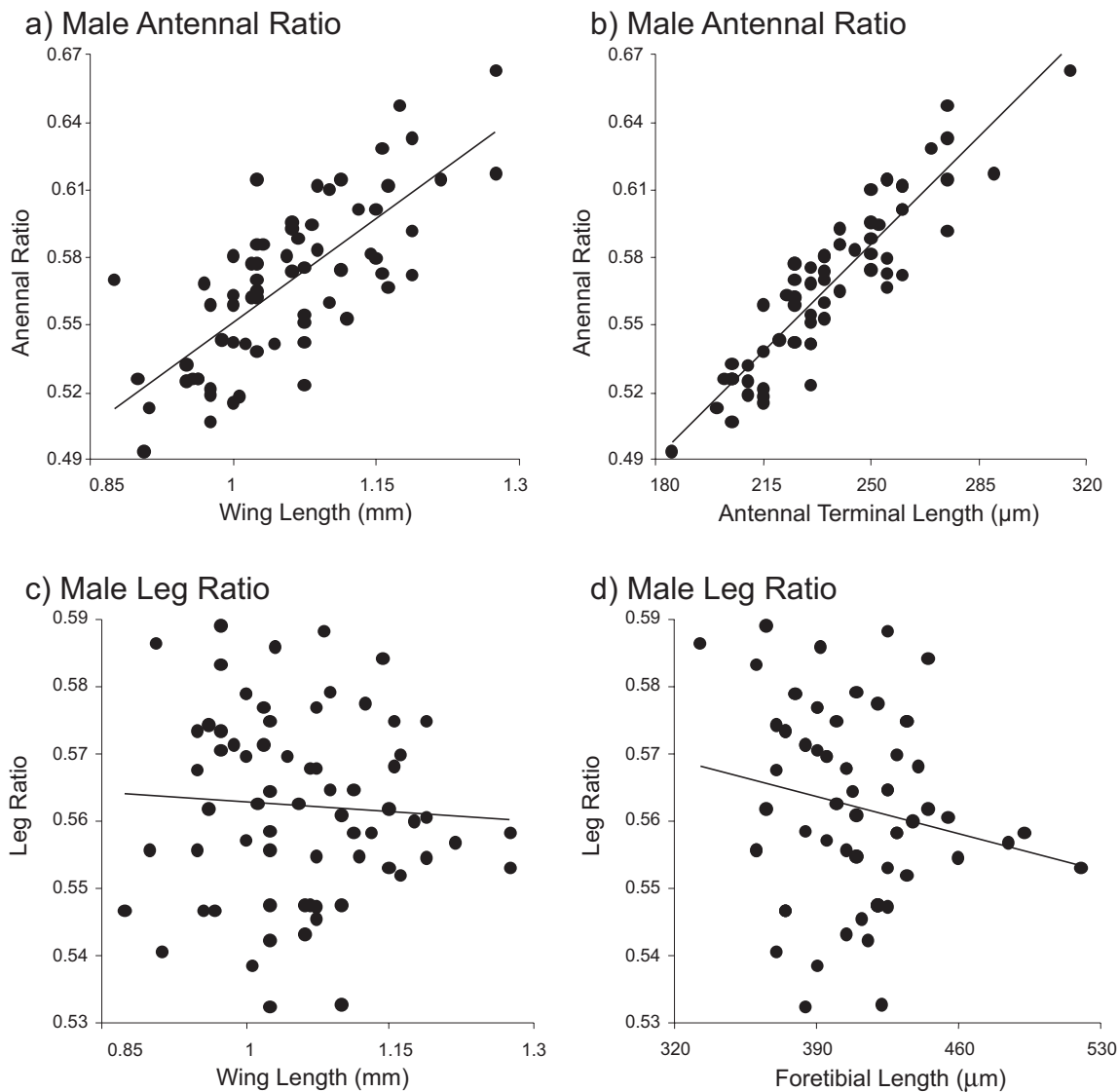
**Table 5.** Wild-caught *E. martini* adult males from the tropical population.

Ratio	Fitted against wing length					Fitted against an alternative size measure*				
	<i>n</i>	Slope	<i>r</i> <sup>2</sup>	<i>F</i>	<i>p</i>	<i>n</i>	Slope	<i>r</i> <sup>2</sup>	<i>F</i>	<i>p</i>
Antennal	67	$3.07 \times 10^{-4}$	0.56	81.69	<0.001	67	$1.37 \times 10^{-3}$	0.82	296.50	<0.001
Leg	68	$-9.28 \times 10^{-6}$	0.00	0.23	0.631	68	$-7.43 \times 10^{-5}$	0.03	2.19	0.144
BV	68	$-4.17 \times 10^{-4}$	0.26	23.12	<0.001	68	$-9.54 \times 10^{-4}$	0.19	15.85	<0.001
SV	68	$-4.46 \times 10^{-5}$	0.00	0.20	0.656	68	$6.46 \times 10^{-6}$	0.00	0.00	0.981
Wing	67	$5.71 \times 10^{-5}$	0.02	1.34	0.251	67	$1.95 \times 10^{-4}$	0.06	4.17	0.045

**Note:** Regression analyses of relationships between size (wing length and alternative size measures) and ratios.

\*Alternative size measures are as follows: antennal ratio (AR in the text) fitted against antennal terminal segment length; leg (LR in the text), BV, and SV ratios fitted against foretibial length; wing ratio fitted against Cu-vein length.

**Fig. 7.** Wild-caught tropical *E. martini*: antennal ratio plotted against (a) wing length and (b) antennal terminal segment length; leg ratio (LR in the text) plotted against (c) wing length and (d) foretibial length. Regression results: (a) *p* < 0.001; (b) *p* < 0.001; (c) *p* = 0.631; (d) *p* = 0.144. See Table 5 for the complete regression results.

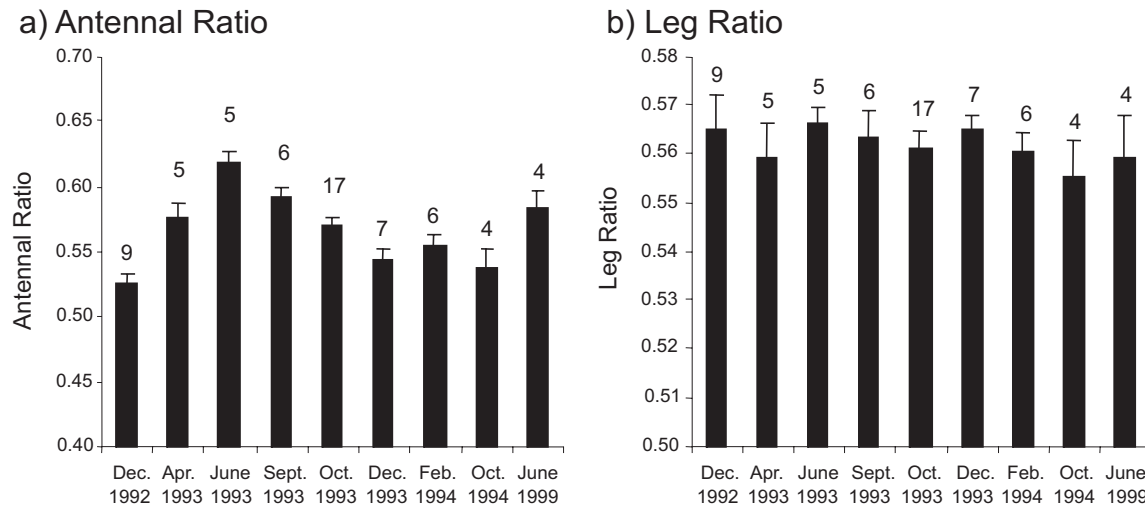


the relationship was reversed, with variation in relationships within, but no divergence between, populations. These contradictory results may relate to the differences in structure of the female antennae between the species, and perhaps also to the lack of variation in the short terminal antennal segment relative to total antennal or wing lengths. Regardless, sizes

of different traits apparently change at different rates relative to one another (e.g., antennal terminal segment size varies much less within populations than wing length).

Arguably, the species we studied may be atypical, given their geographic location (Australia) and widespread distribution (over 1800 km) with associated broad range of devel-

**Fig. 8.** Wild-caught tropical *E. martini*: variation in male (a) antennal ratio (AR in the text) and (b) leg ratio (LR in the text) with sample date. The bars are means  $\pm$  SE, with the number of observations indicated above each bar. ANOVA results — (a) antennal ratio:  $F_{[8,58]} = 11.39$ ,  $p < 0.001$ ; (b) leg ratio:  $F_{[8,58]} = 0.38$ ,  $p = 0.93$ .



opmental temperatures (<7 to >22 °C), and avoltinism. Indeed, elsewhere we argue that these species and other Australian Chironomidae are more mesothermal than stenothermal, with correspondingly wider distributions, as a consequence of the highly variable Australian aquatic environment (McKie et al. 2004). However, similarly wide ranges exist among North American and western European species, and it remains to be seen if natural populations and (or) experimental exposure to a wide temperature range reveal comparable morphometric variation. Nonetheless, description of apparently geographically isolated and (or) anomalous-sized adult individuals as novel taxa based on mensural features ought to be treated with care. The use of such ratios as characters (and variable states) in phylogenetic reconstruction is particularly problematic, as recognized by Cranston and Oliver (1988) and elaborated upon by Cranston and Humphries (1988). Outliers in our data for both laboratory and field data are genuine — there was no misidentification (assessed across multiple life stages in the laboratory study), and re-measurement confirmed the results, which fit trends derived from the main mass of data. These large animals illustrate extreme variability in nature and whose morphometrics could lead to injudicious recognition as differentiated taxa.

Initial discussions by Edwards (1929, 1931) of AR and LR showed awareness of variation. For dry unmounted specimens, Edwards suggested that the degree of variation exceeded the probable error of observations, and thus he estimated rather than measured characters from such specimens. Other sources of mensural variation include the “concertina-like” expansion and contraction of the membranes between sclerotized portions of segmental or pseudosegmental structures such as the antennal flagellomeres (and perhaps even more so for the palpal segments that we have not addressed), inconsistent curvature in longer leg segments, and indistinctness in the precise boundaries of wing veins. Despite this, subsequent authors, notably Schlee (1966) stressed accuracy and precision of measurement. Modern slide-mounted specimens undoubtedly allow better visibility and confidence in measurement accuracy, but this cannot ad-

dress the problem of allometric variation. Caution is required in the use of so-called diagnostic ratios (and size-related counts) of features for taxon discrimination without regard for the allometry demonstrated here. Unfortunately, the allometric relationships identified for our study species cannot readily be extrapolated from one species to another, one ratio to another, or from one sex to the other. Our results also indicate that laboratory-reared individuals for taxonomic purposes will be affected by ambient development temperature and may fail to represent natural variation in either overall size or morphological ratios.

Ratios are not independent measures of species distinctiveness, but neither is any other character, although presence/absence characters may be less vulnerable than division of continua. Multiple comparisons can cluster individuals and allow interpretation, but rarely have adequate samples of true morphological diversity been obtained. Nonmeristic morphology (such as shapes) might seem to assist with environmentally induced meristic variation, but, most perturbingly, Kobayashi (1998) showed allometric (nonlinear) change in an otherwise “highly reliable” indicator of species identity — the male hypopygium. Evidently, a more conservative approach to future morphological species delimitation is required, together with incorporation of whole life-history information to enlighten species concepts, accompanied by molecular studies to test existing morphological segregates, especially of widespread taxa.

### Ecological implications

For our study species, development in cooler water resulted in larger adults and disproportionately longer apical antennal and proximal leg segments. The tightness of these relationships indicates that some biological function may be associated with these ratios, favouring the maintenance of phenotypic plasticity. Pagast (1947) stated that the BV ratio “expresses the ratio of those leg segments that are off the ground to those that are normally resting on the ground as a ‘sole of the foot’. The higher the value of the ratio, the longer are the parts of the leg that are off the ground ...”. We can provide no explanation of our somewhat counter-

intuitive finding of the allometric nature of the BV in which a bigger animal has relatively less of its legs in contact with the ground (scenarios relating to thermoregulation or mating behaviour would be pure speculation).

Regarding the antenna, the Johnston's organs located in the pedicel (the 2nd antennal segment) are receptors (Zhantiev and Fyodorova 1999) that respond to movements of the flagellum (the plumose, pseudosegmented 3rd antennal segment). This sound reception in male Chironomidae allows detection of wing-beat frequency of approaching female conspecifics (Säwedal and Hall 1979; Hirabayashi and Nakamoto 2001), and perhaps also facilitates the maintenance of coherent male mating swarms. Apparently the female (which has a less swollen pedicel than the male) does not detect the male swarm acoustically (Römer 1971), and therefore locates the site by responding to the same visual clues (markers) as the males (Säwedal and Hall 1979). Wing-beat frequencies are invariant with adult age but are lower in females (Römer and Rosin 1969; Römer 1971), and in both sexes correlate with ambient temperature (Ogawa 1992). Published relationships range from 8 to 21 Hz/°C (Säwedal and Hall 1979; Ogawa and Sato 1993) and a multi-species regression showed the most attractive frequency changed at 10 Hz/°C (Hirabayashi and Nakamoto 2001). Furthermore, a correlated seasonal change in frequency of (female-simulating) wing beat that maximally lures conspecific males to traps has been demonstrated (Ogawa 1992; Ogawa and Sato 1993; Hirabayashi and Nakamoto 2001). Although these authors re-examined the wing length of females, finding no correlation with variation in wing-beat frequency, relationships between development temperature and antennal morphometrics were untested.

Our data indicate that cohorts (generations) developing in seasonally cyclical but highly temperature-buffered waters will exhibit allometric plasticity in antennal ratio. This seems problematical, as it is unclear if a variably sized antenna can consistently "tune" to the wing-beat frequency used in species and mate recognition when the ratio of its constituent parts is temperature-dependent. That the feature is under selection for accuracy and rapidity of response is evident from the requirement for the emerged male to locate aurally the female as she approaches the swarm (e.g., McLachlan and Cant 1995). Perhaps the maintenance of variation in this ratio allows adult males to emerge with antennae appropriately tuned to function at ambient aerial temperatures because of their aquatic developmental temperature. The maintenance of variation in this ratio may allow adult males to emerge with antennae appropriately tuned to function at ambient terrestrial temperatures according to their aquatic developmental temperature, although the highly buffered nature of aquatic thermal regimes, which weakens correlation between the temperatures of adjacent aquatic and terrestrial environments (Withers 1992), could reduce the accuracy of such tuning. Such possibilities render the intriguing allometries underlying the morphological ratios of our study species worthy of further investigation by systematists and ecologists alike.

## Acknowledgements

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Zoe Rosser (Department of Zoology and Tropical Ecology, James Cook University) for permission to sort and dissect adult chironomids from light-trap samples collected as part of her doctoral research. Jon Martin (University of Melbourne) kindly furnished us with raw size data for *C. tentans* and gave us permission for its reanalysis. Martin Spies (Zoologische Staatssammlung München, Germany) kindly translated Pagast's original comments. We thank the appropriate Australian authorities for permission to collect our subjects. The senior author was funded by the Rainforest CRC (Cooperative Research Centre) and an Australian Postgraduate Award with stipend. This study was presented at the XV International Symposium on Chironomidae, Minneapolis, Minnesota, in August 2003.

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