

ECOLOGY, BEHAVIOR AND BIONOMICS

A Test of Allen's Rule in Ectotherms: The Case of Two South American Melanopline Grasshoppers (Orthoptera: Acrididae) with Partially Overlapping Geographic Ranges

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Un Test de la Regla de Allen en Ectotermos: El Caso de dos Saltamontes Melanoplinos (Orthoptera: Acrididae) Sudamericanos con Distribuciones Geográficas parcialmente Solapadas

RESUMEN - Estudiamos la variación geográfica de tres caracteres morfométricos en relación al tamaño corporal, en dos saltamontes sudamericanos (Acrididae), *Dichroplus vittatus* Bruner y *D. pratensis* Bruner, para verificar la regla de Allen en ectotermos. Ambas especies siguen la regla de Bergmann inversa por variación latitudinal y/o altitudinal en el tiempo disponible para crecimiento y reproducción; entonces la variación de proporciones de partes salientes del cuerpo, obedecería a crecimiento alométrico diferencial en diferentes áreas geográficas. Alternativamente, podría reflejar variación alleniana relacionada a termorregulación. Las proporciones corporales se estudiaron por análisis de correlación/regresión con variables geográficas y climáticas. En *D. pratensis*, las proporciones corporales aumentaron con la latitud, y decrecieron con la altitud. Esto probablemente obedezca a efectos del balance hídrico y la estacionalidad sobre el tamaño corporal final, y al crecimiento alométrico de los tres caracteres independientemente de la termorregulación. En *D. vittatus*, se observó una tendencia generalmente no significativa al decrecimiento de las proporciones de los tres caracteres con el incremento de latitud. Sin embargo, también es probable que el gradiente ambiental responda a factores de estacionalidad que afectan la duración de la estación de crecimiento y, consecuentemente, el tamaño corporal y sus relaciones alométricas. Concluimos que las relaciones alométricas en la distribución geográfica de proporciones corporales de *D. pratensis* y *D. vittatus* no siguen la regla de Allen en el sentido de la termorregulación, y son el resultado de variables que determinan la duración de la estación de crecimiento y el crecimiento alométrico de diferentes partes del cuerpo.

PALABRAS-CLAVE: Alometría, *Dichroplus*, proporción corporal, tamaño corporal, variación geográfica

ABSTRACT - We studied the geographic variation of three morphometric characters in relation to body size in two South American grasshoppers (Acrididae), *Dichroplus vittatus* Bruner and *D. pratensis* Bruner to test Allen's rule in these ectotherms. Since both species follow the converse to Bergmann's rule owing to latitudinal and/or altitudinal variation in time available for growth and reproduction, geographic variation in body size proportions of protruding parts may obey to differential allometric growth in different geographic areas. Alternatively, it could reflect true Allenian variation related to thermoregulation. Body proportions were studied by correlation/regression analyses with geographic and climatic variables. In *D. pratensis*, body proportions increased with latitude and decreased with altitude. These results probably obey to the effects of water balance and seasonality on final body size, and on the allometric growth of the three studied characters not being related to thermoregulation. In *D. vittatus*, a generally non-significant trend towards the decrease of the mean proportions of all three characters with increasing latitude was observed. Nevertheless, also in this species, it is probable that the environmental gradient responds to seasonality factors (although not to water balance) that affect the length of growing season and, in consequence, body size and its allometric relationships. We conclude that the regularities in the geographic distribution of body proportions of *D. pratensis* and *D. vittatus* do not follow Allen's rule in the sense of thermoregulation, and result from variables that determine growing season length and the allometric growth of different body parts.

KEY WORDS: Allometry, body proportion, body size, *Dichroplus*, geographic variation

Body size and body size proportions tend to follow some ecogeographical patterns, such as Bergmann's or Allen's rules (Bergmann 1847, Allen 1877). Those empirical postulates were originally formulated for endotherms but subsequent work showed that ectotherms, either vertebrate or invertebrate, may show geographic body size patterns that sometimes do agree with the rules' predictions (Ray 1960; Blanckenhorn & Demont 2004; Bidau & Martí 2007a,b).

The literature on ecogeographical rules both in endotherms and ectotherms is abundant and the subject has received considerable attention in recent years (Blackburn *et al.* 1999, Ashton 2001, Millien *et al.* 2006). Regarding body size patterns, most of the work has been devoted to the analysis of intra- and interspecific "Bergmannian" patterns, while Allen's rule, although directly related to the former, has traditionally received little attention.

Allen's rule (Allen 1877) is the generalization that the protruding parts of an endothermic animal's body, such as tail, ears and limbs, are shorter in animals from cold parts of the species range than from warmer parts (Mayr 1942). The gradient in body proportions result from allometric growth of protruding body parts in different parts of the range; this, usually generates latitudinal and/or altitudinal trends in body proportions, so that protruding parts become relatively shorter at higher latitudes and altitudes. These gradients may be coincident with geographic trends in total body mass or size according to Bergmann's rule (Bergmann 1847) which states that body size of organisms living in cooler climates tend to be larger than those inhabiting warmer areas. Allen's rule has been demonstrated for a limited number of endothermic taxa (Griffing 1974, Cartar & Morrison 2005, Yom-Tov *et al.* 2006).

Although many studies have demonstrated geographic and temporal body size and allometric patterns in ectotherms, both vertebrate and invertebrate (Mousseau 1997, Ashton 2002, Ashton & Feldman 2003), the factors underlying these trends may not be the same as those for warm-blooded organisms. Both Allen's and Bergmann's rules in their origins, postulated temperature as the main factor behind body size clines in endothermic vertebrates (mammals and birds). However, especially for Bergmann's rule which has been much more thoroughly explored than Allen's rule, a number of alternative explanations for geographic body size variation have been proposed (Ashton *et al.* 2000, Meiri & Dayan 2003). The most important alternative hypotheses are: 1. Body size clines could result from a need to reduce internal heat loading in warmer environments rather than minimize heat loss in cooler ones (McNab 1979); 2. Changes in insulation could be more important than changes in body mass to conserve internal heat (Geist 1987); 3. Larger animals are found in more seasonal environments because they show higher fasting endurance (Lindstedt & Boyce 1985, Millar & Hickling 1990); 4. Primary productivity may influence body size by its correlation with abundance and quality of food items (Rosenzweig 1968); 5. Larger size may be important for reducing predation or, alternatively, smaller size could reduce inter-specific competition (Damuth 1993); 6. Selection may act on life-history characteristics that are correlated with body size (Ashton *et al.* 2000).

In the case of ectotherms either invertebrate or vertebrate, several hypotheses concerning body size variation have also been proposed reflecting the evidence that some groups follow Bergmann's and Allen's rules, some show converse or compound patterns, and some do not show any pattern at all. Converse Bergmannian clines are much more frequent in ectotherms than in endotherms, especially in insects (Brennan & Fairbairn 1995, Blanckenhorn & Demont 2004, Bidau & Martí 2007a,b). Body size in insects probably obeys to different ecophysiological factors and evolutionary pressures than those in endotherms. Several authors have proposed that size clines result from climatic selection on the duration of egg and nymphal development which indirectly affects body size and can produce different geographic patterns according to the nature of the life cycle (Masaki 1967, 1978; Roff 1980).

We investigated the geographic variation of the proportions of protruding body parts, in two species of grasshoppers of the genus *Dichroplus* Stål (Orthoptera: Acrididae: Melanoplinae) which have very wide and overlapping latitudinal, longitudinal and altitudinal distribution ranges and, consequently, are exposed to a great variation of environmental factors. Both species follow the converse to Bergmann's rule and show pronounced but contrasting (on geographical grounds) sexual size dimorphism (Bidau & Martí 2007b, in press - a,b).

The main objective of this paper was to test the predictions of Allen's rule in both species and identify factors involved in the change of body proportions. Our main hypothesis was that, since both grasshopper species follow the converse to Bergmann's rule owing to latitudinal and/or altitudinal variation in time available for growth and reproduction (Bidau & Martí 2007a, b, in press - c), geographic variation in body size proportions of protruding parts may obey to differential allometric growth in different geographic areas. Alternatively, it could reflect true Allenian variation related to thermoregulation. Thus, we considered a series of climatic factors including temperature, precipitation, evapotranspiration and water balance that could influence the growth of protruding body parts in relation to total body size.

Materials and Methods

Grasshopper species and morphometric data. Twenty five samples of adult *D. pratensis* Bruner (343 males, 352 females) were collected at localities from Argentina spanning 22° of latitude and 0 to 2474 m elevation during February and March, 2001 (Fig.1). Population samples of adult *D. vittatus* Bruner were obtained at 19 Argentine localities (190 males and 174 females) spanning almost 20° of latitude and 36 m to 2758 m above sea level during February and March, 2001 (Fig. 1).

Morphological data corresponding to the mentioned samples have been published elsewhere (Bidau & Martí 2007a,b, in press - c). Based on morphometric external characters of preserved specimens obtained using a precision micrometer (0.01 mm) (Bidau & Martí 2007a, b) we

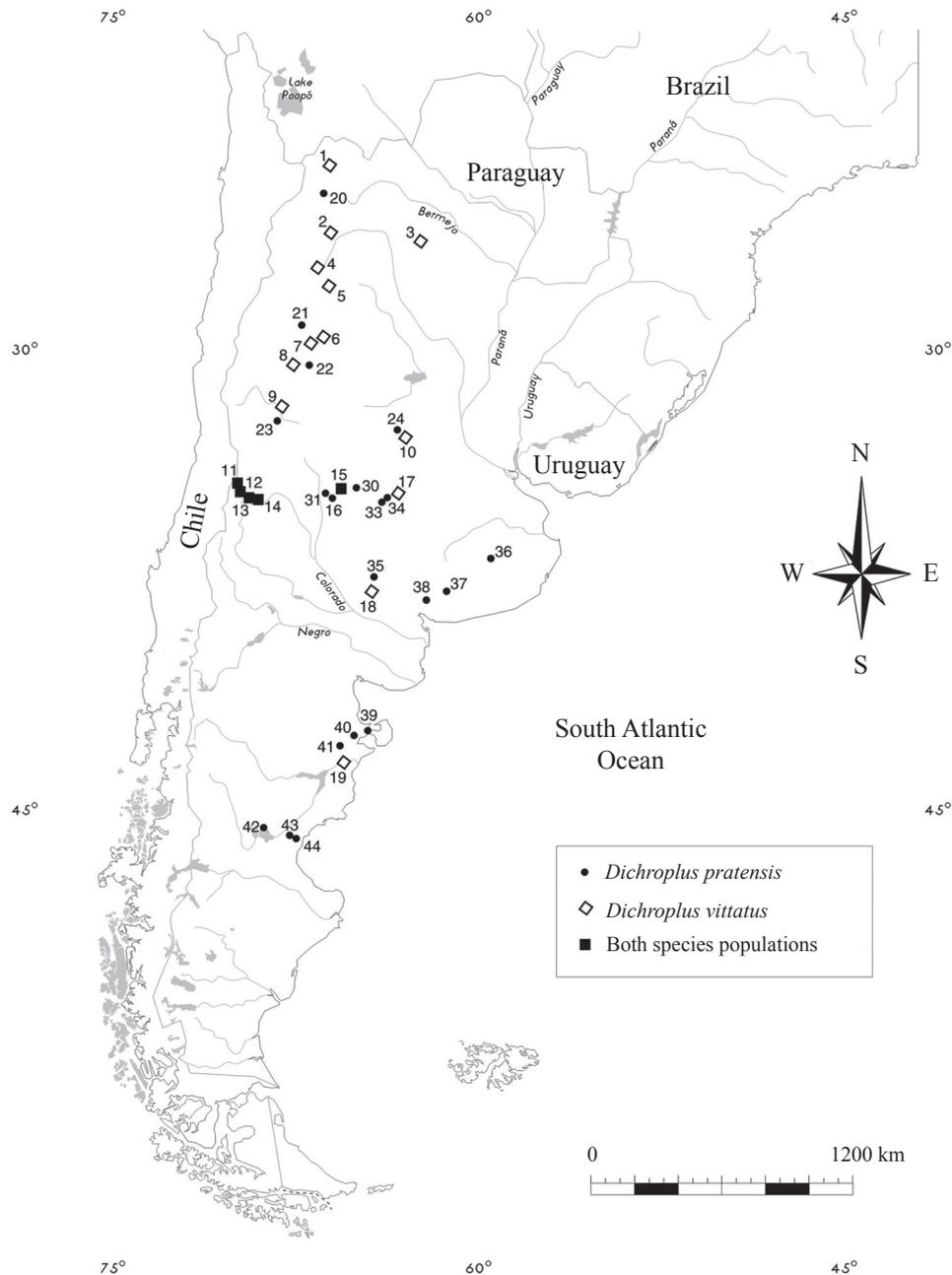


Fig. 1. Geographic distribution of the *Dichroplus pratensis* and *D. vittatus* samples analysed in this paper. Numbers correspond to those localities in the map. For each point, geographic coordinates (all S/W) in degrees and minutes, are indicated as well as altitude in meters above sea level. 1. Huacalera: 23° 26' / 65° 21', 2758; 2. La Viña: 25° 28' / 65° 35', 1265; 3. Santiago del Estero: 26° 01' / 62° 22', 174; 4. Colalao del Valle: 26° 22' / 65° 57', 1662; 5. Tafi del Valle: 26° 52' / 65° 43', 2014; 6. Miraflores: 28° 36' / 65° 41', 537; 7. Chumbicha: 28° 52' / 66° 14', 376; 8. La Rioja: 29° 05' / 66° 40', 521; 9. Valle Fértil: 30° 38' / 67° 27', 828; 10. Villa del Rosario: 31° 34' / 63° 32', 248; 11. Guido: 32° 52' / 69° 15', 2099; 12. Potrerillos: 32° 57' / 69° 11', 1469; 13. Cacheuta: 33° 02' / 69° 07', 1310; 14. Compuertas: 33° 03' / 69° 04', 1063; 15. La Punilla: 33° 08' / 65° 05', 942; 16. El Morro: 33° 13' / 65° 29', 993; 17. Olmos: 33° 30' / 63° 10', 117; 18. Toay: 36° 40' / 64° 21', 174; 19. Playa Unión: 43° 04' / 65° 03', 36; 20. Volcán: 23° 55' / 65° 27' / 2474; 21. Estación Mazán: 28° 44' / 66° 29', 646; 22. Carrizal: 28° 54' / 67° 33', 522; 23. Las Juntas: 30° 41' / 67° 35', 1203; 24. Cañada Machado: 31° 28' / 63° 35', 314; 25. Guido: 32° 52' / 69° 15', 2099; 26. Potrerillos: 32° 56' / 69° 11', 1469; 27. Cacheuta: 33° 02' / 69° 07', 1310; 28. Compuertas: 33° 03' / 69° 04', 1063; 29. La Punilla: 33° 08' / 65° 05', 942; 30. Achiras: 33° 09' / 65° 00', 838; 31. Saladillo: 33° 13' / 65° 52', 840; 32. El Morro: 33° 13' / 65° 29', 993; 33. La Granja: 33° 30' / 63° 17', 577; 34. Manantiales: 33° 33' / 63° 20', 580; 35. Don Tomás: 36° 41' / 64° 20', 175; 36. Olavarría: 36° 55' / 60° 17', 162; 37. Coronel Pringles: 37° 59' / 61° 22', 846; 38. Cerro Ceferino: 38° 06' / 61° 48', 853; 39. Istmo Ameghino: 42° 30' / 64° 32', 55; 40. Puerto Madryn: 42° 49' / 65° 03', 18; 41. Km 1430. Hwy 3: 43° 04' / 65° 12', 36; 42. Lago Musters: 45° 30' / 69° 08', 261; 43. Diadema Argentina: 45° 47' / 67° 40', 326; 44. Villa Rada Tilly: 45° 57' / 67° 32', 0. Black circles: *D. pratensis*; lozenges: *D. vittatus*; black squares: localities where sympatry between *D. pratensis* and *D. vittatus* was verified.

calculated for each population of each species, and separately for each sex, the proportions with respect to mean total body length of (a) mean length of left hind femur (F3L), (b) mean length of left hind tibia (T3L) and, (c) mean length of tegmina (TeL). We calculated those proportions since they represent the most conspicuous protruding parts that can be measured with confidence and less error. All measurements were log-transformed previously to the calculation of the respective proportions.

Geographic independent variables. Latitude (LAT) was included as an independent variable since a negative correlation between a given body proportion and latitude or the lack of it, indicates if Allen's rule applies or not to a certain organism or taxon. Longitude (LON) was considered because several climatic variables show strong longitudinal gradients in the surveyed area (see Results). Altitude (ALT) in metres above sea level was determined for all localities and was included because frequently, an increase in elevation is ecologically equivalent to an increase in latitude (Wigginton & Dobson 1999). However, this kind of one-dimensional analyses have no explanatory power (Hawkins & Diniz Filho 2004), since size clines (e.g. Bergmannian clines) may obey to multiple selection pressures that are not only dependent on temperature constraints but also on other climatic and biotic factors that could influence body size as explained above (Jones *et al.* 2005). This may apply also to Allenian clines, therefore other independent variables were considered as follows.

Environmental independent variables. Climatic data for all samples were obtained from the Cramer & Leemans (2001) database in which mean monthly values of ambient temperature and precipitation covering a 30-year period are recorded with an accuracy of 30 min. Mean annual temperature (TMEAN) was used as an independent variable since Allen's rule (Allen 1877) originally relied on a thermoregulation hypotheses. Total annual precipitation (PANNU) was included because humidity has been hypothesized as relevant in the generation of geographic body size clines (James 1970, 1999; Aldrich & James 1991). In areas of greater climatic seasonality body mass tends to be higher than in less seasonal regions (the fasting endurance hypothesis) (Boyce 1979, Lindstedt & Boyce 1985, Chown & Klok 2003) and thus, could be also involved in changes in body proportions. To estimate seasonality, the annual variability of the climatic factors was calculated. Annual variability of temperature was estimated through the coefficient of variation ($CV = SD * 100 / \bar{x}$) (CVT; where \bar{x} in this case, is the mean annual temperature of each sampled locality, and SD its standard deviation) and the difference between average maximum and minimum annual temperatures (TM-m). SD of TMEAN was obtained from Cramer and Leemans (2001). Variability of precipitation was assessed by the CV of mean annual precipitation (CVP, calculated from mean monthly precipitation and its SD) and the difference between maximum and minimum average monthly precipitation (PM-m). Precipitation data were also taken from Cramer and Leemans (2001).

A further hypothesis pertaining body size clines is that of Rosenzweig (1968), who proposes that body size is positively

correlated with primary productivity and with some correlates as actual (AET) and potential (PET) evapotranspiration (Olalla-Tárraga *et al.* 2006, Rodríguez *et al.* 2006). AET, an estimator of primary productivity (calculated by the Thornthwaite formula), PET, a measure of ambient energy (calculated by the Priestley-Taylor equation), and Water Balance (WB) were obtained for each locality. We used vectors, databases and maps for AET, PET and WB from Ahn & Tateishi (1994a, b). Data analysis was performed with the Geomatica FreeView V. 10.0 software by PCI Geomatics, Ontario, Canada (www.pcigeomatics.com). All data are in mm/year. Mean annual AET, PET and WB values were calculated for each sampled locality.

Statistic analysis. We used SPSS® for Windows® (Statistical Package for the Social Sciences) software to perform all statistical tests. Normality of data was estimated by the Kolmogorov-Smirnov test (with Lilliefors correction). We used correlation and regression analyses to explore the relationships between body proportions of protruding body parts and geographic and environmental variables to identify possible clines. This was done for each studied species and sex. If our basic hypothesis was true we should expect non-Allenian clines of body proportions, related to the allometric consequences of different growth period lengths. Alternatively, if Allenian clines are observed, that is, body size proportions decrease with increasing latitude/altitude or decreasing temperatures, then a thermoregulation hypothesis should be considered. Since most variables departed from normality, Spearman's (non-parametric) correlation coefficients were calculated. Also, due to the amplitude of the geographic distributions of *D. pratensis* and *D. vittatus*, implying wide variation of elevations and climatic factors, most significant relationships were expected to be non-linear. Thus linear and non-linear regressions were performed with the Curvfit command of SPSS 11.01 software.

Results

Correlations between geographic and environmental variables within the studied area. Because geographical trends in body size may be related to environmental variables that covary with LAT, LON and ALT, we explored the relationships between them using non-parametric correlation (Table 1).

Geographical variation of body proportions. Table 2 shows the results of the non-parametric correlations between body size proportions and geographic variables for *D. pratensis* and *D. vittatus* of both sexes. In both species, no simple Allenian pattern was verified when all measurements were considered jointly. In the case of *D. pratensis*, highly significant latitudinal positive trends were observed for all three proportions in both sexes (Table 2; Fig. 2a), while negative trends (most of them statistically significant) occurred when altitude and longitude were the independent variables (Table 2; Fig. 2b-c).

D. vittatus showed a radically different pattern. Except for a significant negative latitudinal trend in female tegmina

Table 1. Spearman correlation coefficients and their statistical significance between environmental and geographical variables for 25 and 19 Argentine localities corresponding to sampled populations of *D. pratensis* and *D. vittatus*, respectively.

Species	Geographic variable	Environmental variables								
		AET	PET	WB	TMEAN	CVT	TM-m	PANNU	CVP	PM-m
<i>D. pratensis</i>	LAT	-0.311	-0.714	0.430	-0.566	0.471	-0.465	-0.182	-0.536	-0.408
		0.130	0.000**	0.032	0.003*	0.016*	0.019	0.384	0.006*	0.043
	LON	-0.638	0.089	-0.718	-0.115	0.284	0.224	-0.697	0.225	-0.400
		0.001**	0.671	0.000**	0.583	0.169	0.282	0.000**	0.279	0.047
	ALT	0.101	0.583	-0.429	0.292	-0.046	0.526	0.044	0.445	0.329
		0.630	0.002*	0.032	0.156	0.826	0.007*	0.833	0.026	0.108
<i>D. vittatus</i>	LAT	-0.104	-0.479	0.729	-0.348	0.506	0.530	0.133	-0.789	-0.449
		0.673	0.038	0.000**	0.144	0.027	0.020	0.586	0.000**	0.054
	LON	-0.732	0.379	-0.458	-0.204	0.281	0.414	-0.799	0.161	-0.480
		0.000**	0.109	0.048	0.401	0.244	0.078	0.000**	0.509	0.037
	ALT	-0.257	0.618	-0.588	-0.373	0.222	-0.228	-0.300	0.398	0.208
		0.289	0.005*	0.008*	0.116	0.361	0.348	0.211	0.091	0.392

The Bonferroni correction was applied to calculate probabilities for the Student's *t*-statistic in both species ($\alpha = 0.016$). LAT, latitude; LON, longitude; ALT, altitude; AET, actual evapotranspiration; PET, potential evapotranspiration; WB, water balance; TMEAN, mean annual temperature; CVT, coefficient of variation of TMEAN; TM-m, difference between maximum and minimum annual temperatures; PANNU, total annual precipitation; CVP, coefficient of variation of PANNU; PM-m, difference between maximum and minimum annual monthly precipitations.

*Correlation is significant at the 0.05 level (two-tailed)
 **Correlation is significant at the 0.01 level (two-tailed)

Table 2. Spearman correlation coefficients between body size proportions and geographic independent variables for male and female data of, respectively, 25 and 19 populations of *D. pratensis* and *D. vittatus*.

Species	Geographic variable	Mean proportion of morphometric character					
		Males			Females		
		F3L/BL	T3L/BL	TeL/BL	F3L/BL	T3L/BL	TeL/BL
Spearman's <i>rho</i> (<i>P</i>)							
<i>D. pratensis</i>	LAT	0.554 (0.004)*	0.552 (0.004)*	0.532 (0.006)*	0.629 (0.001)*	0.661 (0.000)**	0.707 (0.000)**
	LON	-0.551 (0.004)*	-0.565 (0.003)*	-0.692 (0.000)**	-0.250 (0.228)	-0.261 (0.208)	-0.500 (0.011)*
	ALT	-0.468 (0.018)	-0.680 (0.000)**	-0.558 (0.004)*	-0.455 (0.022)	-0.581 (0.002)*	-0.728 (0.000)**
<i>D. vittatus</i>	LAT	0.323 (0.178)	-0.495 (0.031)	-0.340 (0.154)	-0.316 (0.188)	-0.493 (0.032)	-0.600 (0.007)*
	LON	0.395 (0.094)	-0.456 (0.050)	-0.168 (0.491)	-0.407 (0.084)	-0.130 (0.596)	-0.265 (0.273)
	ALT	-0.100 (0.684)	-0.054 (0.828)	0.095 (0.700)	0.062 (0.800)	0.077 (0.753)	0.002 (0.994)

For each species, the Bonferroni correction was applied to calculate probabilities for the Student's *t*-statistic ($\alpha = 0.016$). F3L, length of femur 3; T3L, length of tibia 3; TeL, length of tegmina; BL, total body length; LAT, latitude; LON, longitude; ALT, altitude.

*Correlation is significant at the 0.05 level (two-tailed)
 **Correlation is significant at the 0.01 level (two-tailed)

length and, a marginally significant negative regression between tibia length and latitude, no other body proportions were significantly correlated with geographical variables (Table 2; Fig. 2d,e).

All regressions between body size proportions and geographic variables were non-linear (Fig. 2).

Environmental factors and body proportions. Nine

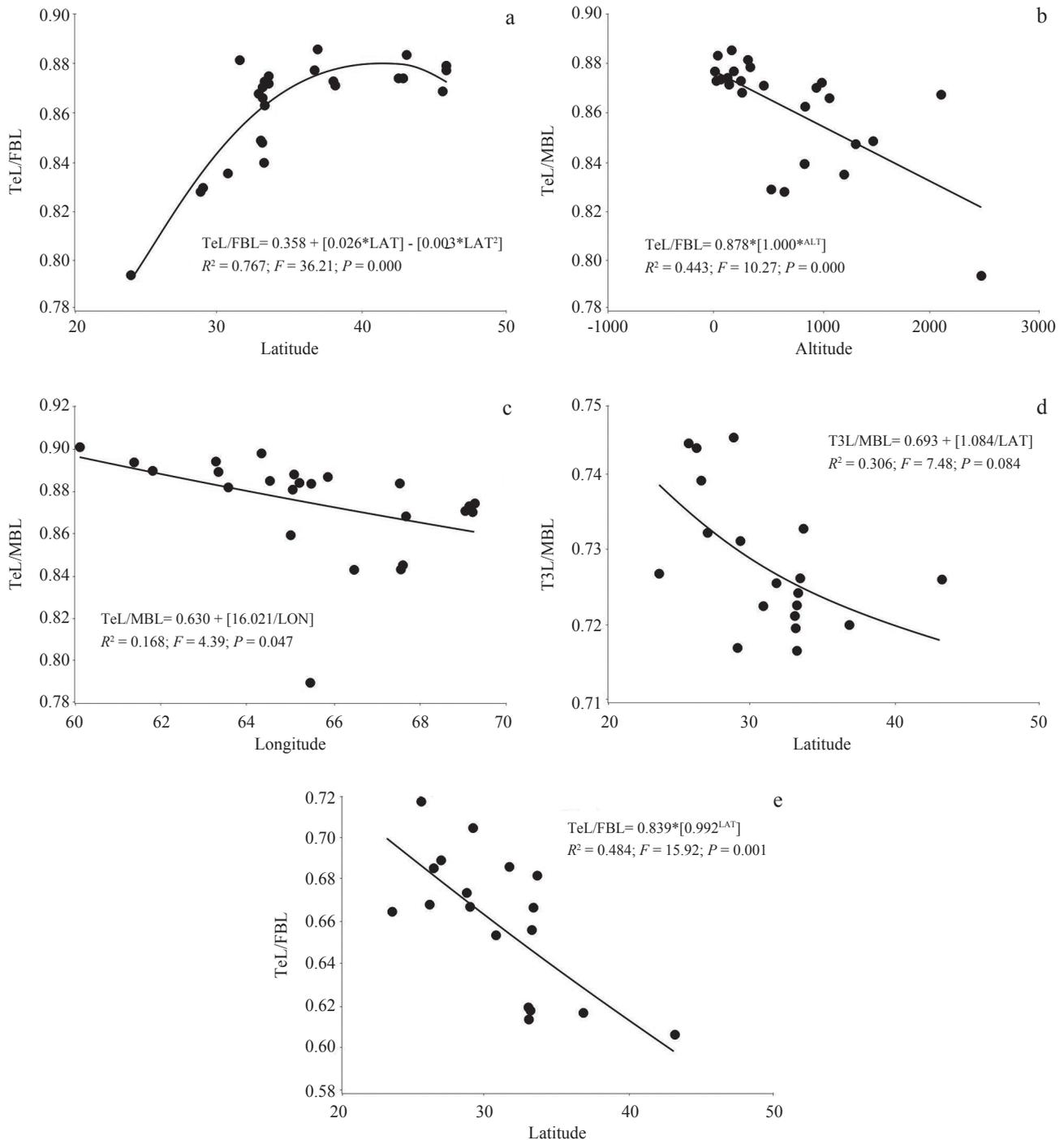


Fig. 2. Relationships between body size proportions relative to total body length (BL), and geographical variables in *Dichroplus pratensis* (a-c) and *D. vittatus* (d,e). a. Proportion of female tegmina length (TeL/FBL) vs latitude (LAT); b. Proportion of female tegmina length (TeL/FBL) vs. altitude (ALT); c. Proportion of male tegmina length (TeL/MBL) vs longitude (LON); d. Proportion of male tibia 3 (T3L/MBL) length vs. LAT; e. Proportion of female tegmina length (TeL/FBL) vs. LAT. In all cases, the regression equation that best fitted the data is shown. R2 = coefficient of determination; F = F statistic; P = probability.

climatic parameters (see Materials and methods) were used as independent variables against body size proportions. Independently of gender and species, evidence of Allen's rule patterns was not found. Only female F3L and T3L proportions showed a significant correlation with mean annual temperature in *D. pratensis*, but these were negative (the converse to Allen's rule). Furthermore, no clear trends were observed when the independent variable was PANNU, although there was a highly significant correlation between male TeL proportion and annual precipitation in *D. pratensis* (Table 3).

Regarding seasonality parameters, only female F3L and T3L proportions, and male T3L proportion, were significantly negatively correlated with TM-m in *D. pratensis* and *D. vittatus* respectively (Table 3; Fig. 3c) while both male and female *D. vittatus* showed significant negative regressions between CVT and TeL (Table 3; Fig. 3e, f).

The correlation of body proportions with AET, PET and WB also yielded contrasting results. In the case of *D. pratensis*, all three female proportions were significantly

negative (Table 3; Fig. 3b). In the case of WB, consistent positive significant correlations were obtained for all proportions in both sexes (Table 3; Fig. 3a), except for female T3L. No significant trends were observed in *D. vittatus* (Table 3).

Non-linearity was almost the rule for body proportion-environmental variable relationships in both species (Fig. 3).

Discussion

No straightforward Allenian patterns were found in the grasshopper species *D. pratensis* and *D. vittatus* within the studied geographic area. *D. pratensis* showed significant latitudinal increases in the proportions of all three measured characters, i.e. they follow the converse to Allen's rule (protruding body parts are proportionally larger at higher latitudes) despite the fact that the species follows the converse to Bergmann's rule (adult body size decreases with increasing

Table 3. Spearman correlation coefficients between body size proportions and environmental independent variables for male and female data of, respectively, 25 and 19 populations of *D. pratensis* and *D. vittatus*.

Species	Climatic variable	Mean proportion of morphometric character					
		Males			Females		
		F3/BL	T3/BL	Te/BL	F3/BL	T3/BL	Te/BL
		Spearman's rho (P)					
<i>D. pratensis</i>	AET	0.320 (0.119)	0.170 (0.417)	0.415 (0.039)	-0.088 (0.675)	-0.204 (0.328)	0.061 (0.773)
	PET	-0.330 (0.103)	-0.493 (0.012)	-0.226 (0.278)	-0.624 (0.001)*	-0.643 (0.001)*	-0.583 (0.002)*
	WB	0.733 (0.000)*	0.571 (0.003)*	0.765 (0.000)*	0.389 (0.055)*	0.306 (0.137)	0.644 (0.001)*
	TMEAN	-0.027 (0.900)	-0.102 (0.627)	0.071 (0.736)	-0.612 (0.001)*	-0.612 (0.001)*	-0.137 (0.513)
	CVT	0.188 (0.369)	-0.138 (0.509)	0.122 (0.562)	0.214 (0.305)	0.155 (0.458)	0.089 (0.673)
	TM-m	-0.093 (0.658)	-0.424 (0.035)	0.012 (0.953)	-0.722 (0.000)*	-0.779 (0.000)*	-0.368 (0.071)
	PANNU	0.411 (0.041)	0.233 (0.263)	0.545 (0.005)*	-0.040 (0.851)	-0.138 (0.512)	0.191 (0.319)
	CVP	-0.291 (0.158)	-0.327 (0.111)	0.468 (0.018)	-0.214 (0.305)	-0.255 (0.218)	-0.477 (0.016)
	PM-m	0.187 (0.371)	0.036 (0.863)	0.176 (0.400)	-0.005 (0.982)	-0.220 (0.291)	-0.132 (0.528)
	<i>D. vittatus</i>	AET	-0.431 (0.065)	0.501 (0.029)	0.440 (0.059)	0.513 (0.025)	0.286 (0.235)
PET		-0.193 (0.428)	0.155 (0.528)	0.193 (0.428)	0.048 (0.844)	-0.159 (0.516)	-0.107 (0.663)
WB		0.068 (0.781)	-0.318 (0.185)	-0.109 (0.657)	-0.159 (0.516)	-0.330 (0.168)	-0.365 (0.124)
TMEAN		-0.104 (0.673)	0.315 (0.189)	0.430 (0.066)	0.071 (0.772)	0.262 (0.278)	0.544 (0.016)
CVT		-0.324 (0.176)	-0.640 (0.003)*	-0.641 (0.003)*	-0.0295 (0.227)	-0.504 (0.028)	-0.775 (0.000)*
TM-m		-0.462 (0.084)	-0.623 (0.004)*	-0.404 (0.086)	-0.330 (0.168)	-0.337 (0.159)	-0.433 (0.064)
PANNU		-0.444 (0.057)	0.411 (0.081)	0.465 (0.045)	0.326 (0.174)	0.087 (0.723)	0.341 (0.154)
CVP		-0.140 (0.567)	0.274 (0.200)	0.274 (0.257)	0.435 (0.063)	0.584 (0.009)	0.544 (0.016)
PM-m		-0.634 (0.004)*	0.702 (0.001)*	0.661 (0.002)*	0.509 (0.026)	0.336 (0.160)	0.610 (0.006)

The Bonferroni correction was applied to calculate probabilities for the Student's *t*-statistic ($\alpha = 0.0056$). F3L, length of femur 3; T3L, length of tibia 3; TeL, length of tegmina; BL, total body length; AET, actual evapotranspiration; PET, potential evapotranspiration; WB, water balance; TMEAN, mean annual temperature; CVT, coefficient of variation of TMEAN; TM-m, difference between maximum and minimum annual temperatures; PANNU, total annual precipitation; CVP, coefficient of variation of PANNU; PM-m, difference between maximum and minimum annual monthly precipitations.

*Correlation is significant at the 0.05 level (two-tailed)

**Correlation is significant at the 0.01 level (two-tailed)

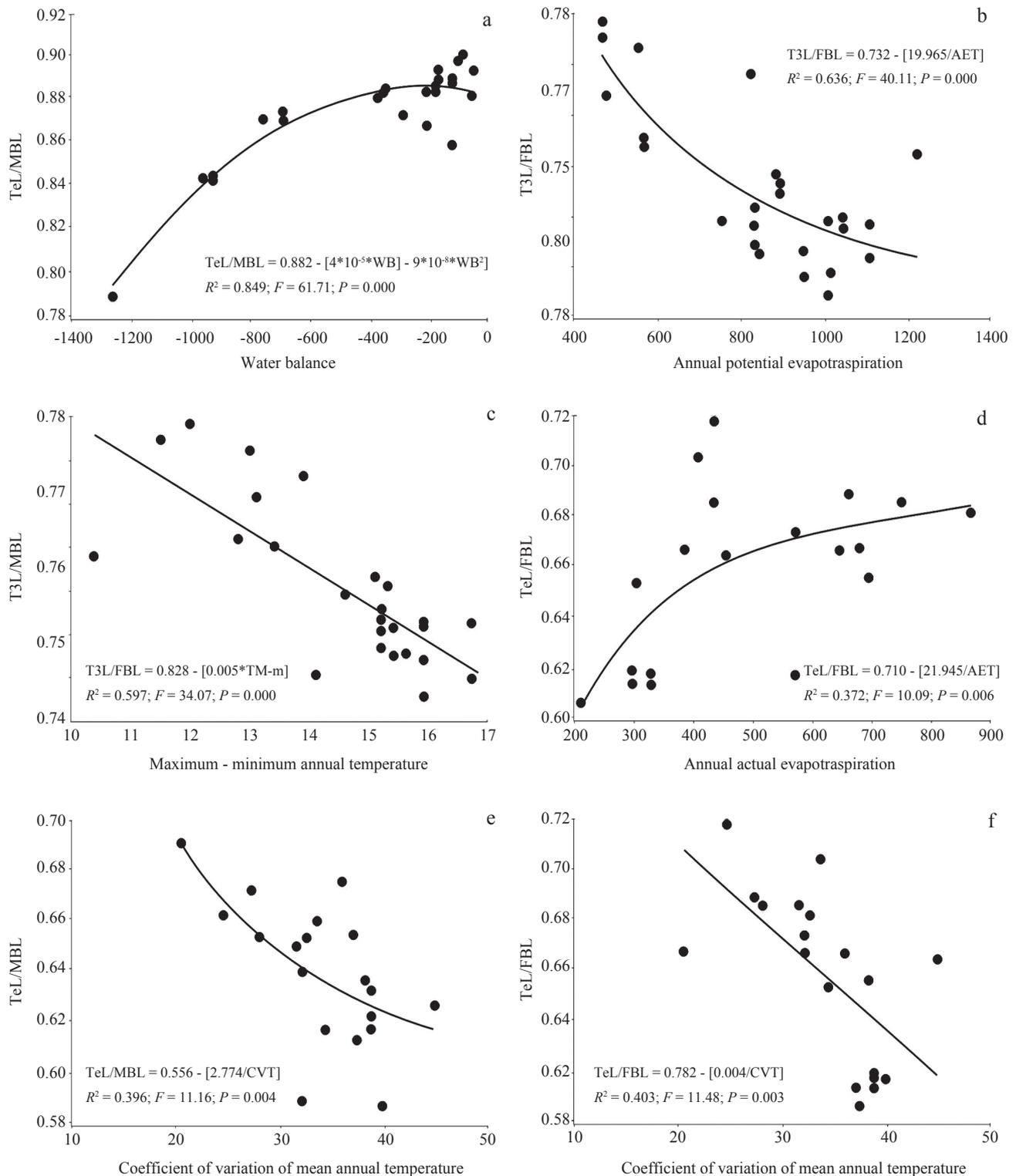


Fig. 3. Relationships between body size proportions relative to total body length (BL) and environmental variables in *Dichroplus pratensis* (a-c) and *D. vittatus* (d-f). a. Proportion of male tegmina length (TeL/MBL) vs. water balance (WB); b. Proportion of female tibia 3 (T3L/FBL) length vs. annual potential evapotranspiration (PET); c. Proportion of female tibia 3 length (T3L/FBL) vs. difference between maximum and minimum annual temperatures (TM-m); d. Proportion of female tegmina length (TeL/FBL) vs. annual actual evapotranspiration (AET); e. Proportion of male tegmina length (TeL/MBL) vs. coefficient of variation of mean annual temperature (CVT); e. Proportion of female tegmina length (TeL/FBL) vs. coefficient of variation of mean annual temperature (CVT). In all cases, the regression equation that best fitted the data, is shown. R^2 = coefficient of determination; F = F statistic; P = probability.

latitude and altitude) (Bidau & Martí 2007a,b, in press - c). However, an opposite pattern was observed altitudinally (negative association between body proportions and the geographic independent variable), which is concordant with Allen's rule. Unexpectedly, the same trends were observed longitudinally (see below). *D. vittatus* practically did not show significant geographic correlations of body size proportions except for a negative latitudinal trend of female tegmina length proportion. Nevertheless, almost all body proportions (except for femur 3 length/total body length of males) showed a negative, albeit non-significant latitudinal decreasing trend in both sexes as shown in Table 2.

Thus, an explanation that takes into account the natural history, climatic correlations and sexual size dimorphism is needed to assess the observed contrasting geographic patterns and differences between species, morphometric traits and sexes.

In a classic paper, Ray (1960) discussed the relevance of Bergmann's and Allen's rules in ectotherm (poikilotherm) organisms based on experimental and field studies of unicellular, arthropod and frog species as well as on previously published data on other taxa. Ray (1960) concluded that both Bergmann's and Allen's rules apply equally to endo- and ectotherms but only at the intraspecific level (*sensu* Mayr 1942). Ray (1960) considered temperature (or latitude, as a proxy) as the only factor related to Allen's rule. His results were particularly clear in the case of *Drosophila* species, which do follow both Bergmann's and Allen's rules where leg proportions with respect to body size tended to decrease with decreasing temperature (Ray 1960).

However, in previous work also discussed in Ray's (1960) paper, Schmidt (1938), Cowles (1945), Park (1949), Hesse *et al.* (1951) and Martof & Humphries (1959) advanced what today is known as a common pattern in ectotherms which is the "converse to Bergmann's rule", that is, in ectotherms body size tends to *decrease* as latitude increases (Mousseau 1997, Blanckenhorn & Demont 2004, Bidau & Martí 2007a). Interestingly, some of the first reported instances of this "inverted" empirical body size pattern seem to support Allen's rule: individuals in the cooler parts of the range tend to be smaller but also to have relatively shorter limbs (Ruibal 1955, Martof & Humphries 1959, Ray 1960). This is not, however, the case of endotherms, where body size usually increases with latitude (with exceptions; see Ashton *et al.* 2000, Meiri & Dayan 2003, Medina *et al.* 2007).

This puzzle may be partially resolved if it is considered that a large number of laboratory experiments have demonstrated that at lower temperatures, most ectotherms tend to grow slower but attain larger size than at higher temperatures (Atkinson 1995, Atkinson & Sibly 1997, Angilletta *et al.* 2004). However, in nature and at the geographical level, this is not always the case.

In univoltine insects of temperate regions such as *D. pratensis* and *D. vittatus*, geographic variation of body size is more related to the available time for embryonic and nymphal development determined by environmental parameters, and also by evolutionary constraints, thus, only indirectly related to latitude or altitude. Furthermore, early emergence and maturation of males (protandry) in these species determinate

smaller sizes in males but also probably different body size proportions (Bidau & Martí 2007a,b, in press - c). Developmental and maturation times certainly depend on environmental factors that are usually correlated with geographic variables although sexual selection may also play a role (Masaki 1978; Bidau & Martí 2007b, in press - b).

The former could explain why water balance appears as a very important factor in relation to the variation of all characters in *D. pratensis* while it seems to be irrelevant in *D. vittatus*. Indeed, WB is positively correlated with latitude and negatively with altitude, for the localities where *D. pratensis* was sampled. These correlations agree in sign with the latitudinal and altitudinal trends in body proportions (non-Allenian and Allenian, respectively). Total body size increases with water balance and the three analyzed morphometric traits show pronounced negative allometry, except for length of tegmina of males which shows isometry (Bidau & Martí 2007a,b). Hence, it is possible that the altitudinal and latitudinal variations of body proportions obey more to general body size variation dependent on water availability, than to an adaptation to mean temperature, as Allen's rule requires. This hypothesis also explains the observed negative longitudinal gradients, since water balance is negatively associated with longitude within the sampled geographic area. Correlations of body proportions with seasonality parameters reinforce the former hypothesis.

It is worth of note that *D. vittatus*, closely related to *D. pratensis* and inhabiting the same general geographic area, shows a radically different behavior regarding the object of this study. Although this species did not show, in general, significant correlations between proportions of protruding body parts and geographic and climatic variables, a recurrent negative latitudinal trend for the three characters was observed in both sexes. This observation would seem to concord with Allen's rule. However, possible climatic predictors of body size proportions are difficult to identify.

Contrary to *D. pratensis*, water balance did not exhibit any relationship with the studied characters but this is not at all surprising: although *D. vittatus* geographic range partially overlaps that of *D. pratensis*, both species are only rarely found sympatrically at the same locality independently of latitude or altitude. *D. vittatus* seems to be better adapted to much more arid environments than *D. pratensis* which could explain its independence from water balance both with respect to body size (Bidau & Martí 2007a) and body proportions.

Parameters of seasonality were relatively good predictors of change in body proportions which were negatively correlated with them. Since higher latitudes tend to be more seasonal, this fact may explain the apparent latitudinal concordance to Allen's rule in *D. vittatus*. It is probable that, as in *D. pratensis*, seasonality affects length of developmental time (shorter developmental time at higher latitudes) and consequently, final body size (Masaki 1967, Bidau & Martí 2007a,b), thus, the latitudinal differences in adult total body length will be reflected in different body proportions since the analyzed body parts show also in this species, negative allometric growth (Bidau & Martí 2007a).

Allen's rule was formally related to temperature and thermoregulation (Allen 1877) and has been usually

considered as a complement to Bergmann's rule (Ashton 2001). However, although the latter has received much support from experimental and biogeographic studies (although reverse patterns are frequent and a number of interpretations other than temperature thermoregulation have been put forward), Allen's rule has received little attention, perhaps because results have been more complicated and conflicting than those of Bergmann's rule. In fact, some authors have considered Allen's rule an exception rather than a rule, at least in endotherms, since protruding parts may be under strong selective pressures other than those relating to thermoregulation (Stevenson 1986). In fact, insects and particularly grasshoppers, thermoregulate behaviorally (Pepper & Hastings 1952, Heinrich 1995, Willott 1997) and probably, owing to the small body size of insects with respect to the majority of endotherms, changes in size of protruding body parts do not add much to body heat conservation on purely geometric grounds (Willott & Hassall 1998).

Therefore, it is concluded that the observed trends in the geographic distribution of body size proportions of *D. pratensis* and *D. vittatus*, which do not obey to Allen's rule and seem to have no relation with body heat conservation, are possibly a reflection of different life histories centered on factors that determinate the length of the growing season for each species, protandry and the allometric growth of body parts resulting from these factors.

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