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Analysis of body size and fecundity in a grasshopper

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Abstract

We used linear regression, nonlinear regression and principal component analysis to examine the relationships among morphology, fecundity, and mating variables for lab-reared adult female *Romalea microptera* (Beauvois) (fam. Romaleidae) grasshoppers. Morphological variables included head width, pronotum length, femur length, adult eclosion mass, maximum mass reached before the 1st oviposition, and maximum mass reached before the 2nd oviposition. Fecundity (= reproductive) variables included clutch size Pod 1, clutch size Pod 2, total eggs Pods 1 + 2, mass Pod 1, mass Pod 2, time between adult eclosion and Pod 1, time between Pod 1 and Pod 2, and time between eclosion and Pod 2. Mating variables included number of matings and age of 1st mating. Most morphological variables were strongly positively correlated, and morphological variables (especially femur length, eclosion mass, and maximum body mass reached prior to oviposition) predicted many fecundity variables. Maximum body mass reached prior to laying Pod 1 was highly correlated with maximum body mass reached prior to laying Pod 2 (r =0 .93), and clutch size Pod 1 predicted clutch size Pod 2 (r = 0.72). However, time to oviposit (= interval between adult eclosion and oviposition) was generally unrelated to body size, body mass, or clutch size or mass. Hence, clutch size and pod mass are strongly determined by body size and mass at adult eclosion, but timing of oviposition is independent of body size and mass at eclosion. The results also suggest that early mating speeds oviposition, but that excessive mating reduces female fecundity, as measured by clutch size.

Key words

Acrididae, Romaleidae, *Romalea microptera*, grasshopper, morphology, morphometrics, clutch size, oviposition, fecundity, reproduction, mating, body mass, size, femur length, egg pod, tradeoffs

Introduction

Body size and mass are important in biology because biological rates, performance, and interactions vary as a function of these morphological characters (McMahon & Bonner 1983, Thornhill & Alcock 1983, Calder 1984, Schmidt-Nielson 1984, LaBarbera 1989, Peters 1989, Reiss 1989, Roff 1992, Stearns 1992, McShea 1998). Because of their strong correlations with performance, body size and mass serve as valuable predictive tools, and thus are often important components in modeling (Livdahl & Sugihara 1984, Peters 1989, Fielding 2004). Because size and mass are more easily measured than many other organismal features (*e.g.*, metabolic rate, caloric content, lifetime fecundity, competitive ability, niche, etc.), biologists often use body size to estimate other characteristics.

In insects, studies show that large females often possess more ovarioles, lay more eggs, lay larger eggs, feed and assimilate nutrients faster, and sometimes reproduce earlier than smaller females (Peters 1989, Roff 1992, Honek 1993, Blanckenhorn 2000, Fox & Czesak 2000, Hodin 2009). In contrast, smaller females often reproduce earlier or faster than large females (Stearns 1992, Kriegbaum 1997, see Blanckenhorn 2000). In addition, research has documented tradeoffs among various life-history features such as egg size, clutch size, time to reproduce, etc. (Roff 1992, Stearns 1992, Fox & Czesak 2000, Hodin 2008).

However, biologists often allege relationships among size, mass, and fecundity, or the existence of tradeoffs, without actual data to support such claims. In addition the literature is replete with assertions that large individuals are more fit than small individuals. However, if large individuals were always more fit, then Earth would be populated only by large species (Blanckenhorn 2000). In short, such claims need to be supported with actual data.

In this paper we quantify the relationships between size/mass and reproductive outcomes in a grasshopper. We correlate morphological parameters of adult female lubber grasshoppers with measurements of fecundity, to determine which morphological parameters best predict reproductive outcomes. We also examine correlations among the morphological features only, and among the reproductive features only, to determine which are, and which are not, related. We wanted to know which single factor or combination of factors best predicted other factors, and which models (linear, quadratic, logistic, cubic, *etc.*) best described these relationships. We subject our data to three analytical methods: simple linear regression, non-linear regression, and principal component analysis.

Materials and methods

Data for this paper were derived from a previously published laboratory study (Walker *et al*. 1999) on the effects of mating and social grouping on fecundity in female Eastern Lubber grasshoppers, *Romalea microptera* (Beauvois). The original experiment consisted of three treatments (see below).

Source and Care of Animals.— Experimental animals originated from a laboratory colony of *Romalea microptera* (Beauvois) (= *guttata*, see Otte 1995) held at Illinois State University (Matuszek & Whitman 2001). This colony was established in 1996 and 1997 from wild animals collected near Copeland, Florida, USA.

On the day females eclosed to adulthood they were weighed, measured for size, and divided into three treatment groups. Individual identification numbers were painted onto the wings of each female. Each treatment was housed in a separate environmental chamber and consisted of 20 females. Treatments were balanced such that

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each treatment had a similar distribution of size and mass. In addition, before assigning animals to treatments, we removed extremely large or small individuals (~ 8% of all animals). This made the treatment groups in the original experiment more homogenous, but it eliminated extremes for our current analysis. All females were fed Romaine lettuce and oatmeal *ad libitum*. Environmental chambers were maintained at a 32:24°C L:D temperature regime and a 14:10 L:D photoperiod. All chambers were calibrated to the same thermometer. Female containers were rotated within incubators daily in order to balance any variation in chamber temperature.

For all treatments, we raised females from the adult molt (Day 0) until they laid their second egg pod (~ Day 49 to 54). For oviposition, females older than 24 d were placed in individual cups containing moist sand for 4 h each day until they laid. Females, along with any feces, were weighed before and after laying to determine the approximate egg-pod mass. Egg pods were dug up and dissected to determine clutch size (= number of eggs).

Treatment 1: isolated virgins.—The Virgin Treatment group served as the control. Virgins were maintained in a "clean room", devoid of other grasshoppers or male odors. Each female was held individually in a ventilated plastic container (1350 cm^3). Cardboard barriers around each container blocked visual stimuli from adjacent females. Hence, females in this treatment were not exposed to male presence, male odor, mating stimuli, or visual or tactile stimuli from conspecifics. Although these females never mated, they developed and laid eggs.

Treatment 2: isolated-mated females.—Treatment 2 females were treated as those of Treatment 1, except they were mated three times: once each on ~ Days 21 and 26 of adulthood, and again after the first oviposition.

Treatment 3: mixed-sex group.—This treatment examined the combined effects of mating and social grouping (including: visual, tactile, and chemical stimuli from constant male and female conspecific association) on female fecundity. It consisted of two 57-L glass terrariums, each containing 10 females and 10 males, held communally.

Data collected.—For all females, we used calipers and a balance to measure adult **morphological variables** (head width at the compound eyes, pronotum length along the dorsal midline, hind femur length, wet mass at adult eclosion, greatest wet mass reached prior to 1st oviposition, and greatest wet mass reached prior to 2nd oviposition), **mating variables** (number and timing of copulations), and **fecundity variables** (clutch size for the 1st and 2nd egg pods, the intervals, in days, from adult eclosion to the 1st oviposition, and from the 1st to the 2nd oviposition, and wet mass lost by females during each oviposition [= pod mass]). Only females that laid eggs were included in the analysis. Twenty-one of the 60 original females were eliminated from the analysis because they died early, did not lay, or exhibited various pathologies. We undertook three analyses: linear regression, nonlinear regression, and principal component analysis.

Linear regressions.— Using the above data set, we first produced simple pair-wise linear regressions of each variable to every other variable, using Microsoft Excel®. The original experiment (Walker *et al.* 1999) demonstrated significant differences among the three treatment groups (designated T1, T2, and T3) in mean age (days) to 1st oviposition (T1 = 34.6 d, T2 = 31.2 d, T3 = 32.1 d) and 2nd oviposition (T1 = 53.8 d, T2 = 49.1 d, and T3 = 48.7 d) (see Walker et al. 1999 for ANOVA statistics). To remove the effects of treatment on time, we adjusted the times for all Treatment 1 and Treatment 3 data, so that the means for the time to 1st pod and 2nd pod for all three treatments were identical to Treatment 2, at 31.2 d and 49.1 d, respectively. For example, we added 3.4 d (the difference between Treatments 1 and 2 in the mean time to lay the 1st pod) to the time to reach the 1st pod to each animal in Treatment 1. Removing the treatment effects on time from the original experiment allowed us to pool the data from all three treatments. These transformed data were only used for time *vs* morphology comparisons. In contrast, we used the original (nonadjusted) time data when regressing mating variables to fecundity variables, or when comparing among fecundity variables, because we assumed that the original treatments did not influence the relationships between the other reproductive variables

Nonlinear regressions.— In order to explore possible nonlinear associations between reproductive characteristics and morphological factors, we used Microsoft Excel to fit various nonlinear models (exponential, power and cubic polynomial) to each of the regressions. We then used Microsoft Excel to calculate the coefficient of determination (R^2) for each model, and selected the model with the highest R^2 value, as the model with the best fit. Because outlying data may inordinately influence R^2, we also tested for outliers using Grubb's test for outliers, which failed to detect outliers in our data (Grubbs 1969 and Stefansky 1972).

Principal component analysis (PCA).— Analyses 1 and 2 (above) are pair-wise: they examine the relationship of a single factor to another single factor. PCA allowed us to consider the effects of multiple factors simultaneously on a single variable (Kachigan 1986). As such, PCA uses more information and usually explains more of the variance in a dependent variable. In principle, two or more highly correlated independent variables contribute little additional information toward understanding a single dependent variable, over that provided by just one of the variables. Consequently, PCA eliminates highly correlated variables and thus focuses on a core set of independent variables that explain a meaningful portion of the total variation in the dataset.

We performed a PCA on our data using SPSS® (SPSS Inc., Chicago). In order to determine which subset of independent variables best explained the total variation of single dependent variables, we first obtained extraction communalities (Kachigan 1986), which estimated the percent variance in each dependent variable accounted for by possible combinations of independent components. We took the variables corresponding to the selected principal components and calculated the fraction of variation explained by each. We then fitted various regression models to the principal factors and performed cluster analysis of variables: cluster analysis is an exploratory tool designed to reveal natural groupings (or clusters) within a dataset that would otherwise not be apparent. This procedure identifies relatively homogeneous groups of variables based on intercorrelation characteristics, by using an algorithm that starts with each variable in a separate cluster and combines clusters until only one is left, based on adjusted partial correlation of the variables (Kachigan 1986). This procedure produces a dendrogram, which is a graphical summary of the cluster solution (see Aldenderfer & Blashfield 1984). Finally, we calculated the C_p statistic (which simultaneously maximizes r^2 and minimizes the number of variables) for all possible

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Table 1. Linear correlation coefficients (r) for various contrasts of grasshopper morphology, fecundity, and mating variables, arranged by significance level for correlation (2-tailed test). Correlations in the right-hand column are the strongest, have the highest significance, and thus have the highest predictive value. See Methods section for explanation of terms. In the contrasts column, "bf." refers to "before". "P" refers to the p-value of the test of significance for the correlation coefficient.

	Correlation coefficient (r)		
Contrasts (Independent × Dependent)	P > 0.05	0.05 > P > 0.001	P < 0.001
Morphology × Morphology			
Head × Pronotum		0.38	
Head × Femur		0.38	
Head × Eclosion mass		0.42	
Head × Max mass bf. pod 1		0.42	
Head × Max mass bf. pod 2		0.36	
Pronotum × Femur			0.55
Pronotum × Eclosion mass			0.66
Pronotum × Max mass bf. pod 1		0.42	
Pronotum × Max mass bf. pod 2		0.36	
Femur × Eclosion mass			0.74
Femur × Max mass bf. pod 1			0.74
Femur × Max mass bf. pod 2			0.66
Eclosion mass × Max mass bf. pod 1			0.77
Eclosion mass × Max mass bf. pod 2			0.70
Max mass bf. Pod 1 × Max mass bf. pod 2			0.93
Morphology × Fecundity			
Head × Clutch size pod 1		0.34	
Head × Time: eclosion - pod 1	-0.113		
Head × Mass pod 1		0.32	
Head × Clutch size pod 2	0.150		
Head × Time interval: pod 1-2	0.100		
Head × Mass pod 2	0.068		
Head × Total eggs pods 1+2		0.34	
Head × Time: eclosion-pod 2	-0.012		
Pronotum × Clutch size pod 1		0.38	
Pronotum × Time: eclosion - pod 1	-0.039		
Pronotum × Mass pod 1	0.250		
Pronotum × Clutch size pod 2	0.149		
Pronotum × Interval: pod 1-2	0.202		
Pronotum × Mass pod 2			0.51
Pronotum × Total eggs pods 1+2		0.37	
Pronotum × Interval: eclosion-pod 2	0.186		
Femur × Clutch size pod 1			0.63
Femur × Time: eclosion - pod 1	-0.093		
Femur × Mass pod 1			0.54
Femur × Clutch size pod 2			0.57
Femur × Time interval: pod 1–2	.039		
Femur × Mass pod 2			0.55
Femur × Total eggs pods 1+2			0.67
Femur × Interval: eclosion-pod 2	-0.024		
Eclosion mass × Clutch size pod 1			0.67
Eclosion mass × Time: eclosion - pod 1	0.019		
Eclosion mass × Mass pod 1			0.53
Eclosion mass × Clutch size pod 2			0.55
Eclosion mass × Interval: pod 1–2	0.084		
Eclosion mass × Mass pod 2			0.52
Eclosion mass × Total eggs pods 1+ 2			0.52
Eclosion mass × Interval: eclosion-pod 2	0.068		
Max mass bf. pod 1 × Clutch size pod 1			0.69
Max mass bf. pod 1 × Time: eclos.-pod 1	-0.104		
Max mass bf. pod 1 × Mass pod 1			0.59
Max mass bf. pod 1 × Clutch size pod 2			0.62
Max mass bf. pod 1 × Interval: pod 1–2	-0.025		
Max mass bf. pod 1 × Max mass < pod 2			0.65
Max mass bf. pod 1 × Total eggs 1+ 2			0.66
Max mass bf. pod 1 × Time Eclos.-pod 2	-0.077		
Max mass bf. pod 2 × Clutch size pod 2			0.70
Max mass bf. pod 2 × Interval: pod 1–2	-0.094		
Max mass bf. pod 2 × Mass pod 2			0.75
Max mass bf. pod 2 × Total eggs 1+2			0.67
Max mass bf. pod 2 × Time: eclos.-pod 2	-0.153		
Fecundity × Fecundity			
Clutch size pod 1 × Time: eclos.- pod 1	-0.236		
Clutch size pod 1 × Mass pod 1			0.68
Clutch size pod 1 × Clutch size pod 2			0.72
Clutch size pod 1 × Interval: pod 1–2	-0.019		
Clutch size pod 1 × Mass pod 2		0.46	
Clutch size pod 1 × Max mass bf. pod 2			0.70
Time: eclos.-pod 1 × Clutch size pod 1	-0.236		
Time: eclos.-pod 1 × Mass pod 1		-0.32	
Time: eclos.-pod 1 × Max mass bf. pod 2	-0.142		
Time: eclos.-pod 1 × Clutch size pod 2	-0.080		
Time: eclos.-pod 1 × Time pod 1 - pod 2	0.280		
Time: eclos.-pod 1 × Mass pod 2	-0.236		
Mass pod 1 × Clutch size pod 1			0.68
Mass pod 1 × Time: eclosion - pod 1		-0.32	
Mass pod 1 × Clutch size pod 2		0.42	
Mass pod 1 × Mass pod 2			0.51
Clutch size pod 2 × Interval: pods 1-2	-.005		
Clutch size pod 2 × Mass pod 2			0.66
Interval: pods 1-2 × Mass pod 2	-0.155		
Total eggs pods 1+2 × Interval: E-2	-0.145		
Mating contrasts			
Treatment 2 only			
Femur length × Age 1st mate	0.351		
Eclosion mass × Age 1st mate	-0.249		
Age 1st mating × Clutch size Pod 1	-0.171		
Age 1st mating × Clutch size Pod 2	-0.252		
Treatment 3 only			
Femur length × Age 1st mate	0.368		
Eclosion mass × Age 1st mate	0.364		
Femur length × # matings bf. Pod 1	-0.448		
Eclosion mass × # matings bf. Pod 1	-0.214		
# matings bf. Pod 1 × Time to Pod 1	0.289		
Age 1st mating × Clutch size Pod 1	0.390		
Age 1st mating × Clutch size Pod 2	-0.250		
Treatments 2 + 3 only			
# matings bf. Pod 1 × Time to Pod 1	0.239		
Age 1st mating × Clutch size Pod 1	0.109		
Age 1st mating × Clutch size Pod 2	-0.121		
All Treatments			
Age 1st mate × Time to Pod 1	0.298		
Age 1st mate × Mass Pod 1	0.178		
Age 1st mate × Mass Pod 2	-0.277		
Age 1st mate × Interval: Pods 1-2	0.243		
Age 1st mate × Time: Eclosion-Pod 2		0.33	
Age of mating bf. Pod 2 × Mass Pod 2	0.176		
Age of mating bf. Pod 2 × Interval: P 1-2		0.35	
Interval: Pod 1-next mating × E to Pod 2		0.39	
# matings bf. Pod 1 × Clutch size Pod 1	-0.317		
# matings bf. Pod 1 × Clutch size Pod 2		-0.36	
# matings bf. Pod 2 × Clutch size Pod 2		-0.45	
Age 1st mating × Clutch size Pod 1	0.120		
Age 1st mating × Clutch size Pod 2	-0.003		

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Table 2. Pairwise contrasts of female grasshopper morphological variables (left-hand column) with fecundity characteristics (top row), showing "best-fit" nonlinear models, and resulting coefficient of determination (R^2) for each contrast. See Methods section for explanation of terms. "bf." refers to "before".

	Maxmass bf. pod 1	N eggs pod 1	Time to pod 1	Maxmass bf. pod 2	N eggs pod 2	Mass pod 1	Mass pod 2	Time E to pod 2	Time pod 1 to pod 2	AdjTime E to pod 1	AdjTime pod 1 to pod 2	AdjTime E to pod 2	Total eggs laid
Head length	Poly- 3 0.19	Poly- 3 0.12	Poly- 3 0.089	Poly- 2 0.13	Poly- 3 0.048	Poly- 3 0.13	Poly- 3 0.014	Poly- 3 0.17	Poly- 3 0.15	Poly- 3 0.16	Poly- 3 0.18	Poly- 3 0.27	Poly- 3 0.13
Pronotum length	Poly- 3 0.52	Poly- 3 0.18	Poly- 3 0.10	Power 0.44	Poly- 3 0.20	Power 0.083	Poly- 3 0.33	Poly- 3 0.097	Poly- 3 0.11	Poly- 3 0.085	Poly- 3 0.17	Poly- 3 0.18	Poly- 3 0.29
Femur length	Power 0.57	Poly- 3 0.43	Poly- 3 0.10	Power 0.44	Poly- 3 0.27	Poly- 3 0.33	Poly- 3 0.33	Poly- 3 0.026	Poly- 3 0.0095	Poly- 3 0.19	Poly- 3 0.035	Poly- 3 0.060	Poly- 3 0.45
Eclosion mass	Poly- 3 0.72	Poly- 3 0.47	Poly- 3 0.12	Poly- 3 0.61	Poly- 3 0.33	Power 0.31	Poly- 3 0.35	Poly- 3 0.082	Poly- 3 0.053	Poly- 3 0.093	Poly- 3 0.042	Poly- 3 0.064	Poly- 3 0.37

combinations of variables, and compared the C_p values against p (the number of variables) (Mallows 1973). The model with the lowest C_p value approximately equal to p is considered the most parsimonious model.

Results

Linear regression.— Table 1 gives correlation coefficients (r) for all 107 pair-wise linear contrasts, and Figure 1 illustrates the linear regressions of a subset of these contrasts. Note that some variables were highly correlated with others; however, time variables showed low correlations with all morphology and fecundity factors, with a trend for negative correlations. Females that mated late, laid their 2nd pod late, and females that had more mates laid smaller clutches; otherwise, mating had little effect on fecundity (Table 1).

Nonlinear regression.— Tables 2 and 3 give the "best-fit" nonlinear model and coefficient of determination value (R^2) for each of the morphology *vs* fecundity contrasts, and for pair-wise comparisons among selected fecundity variables, respectively. Note that the polynomial models are restricted to third order polynomials (Poly-3). As expected, different contrasts are best explained by different models. For example, the maximum wet-body mass reached prior to laying the 1st pod (maxmass bf. pod 1) is best predicted (R^2 = .72) by eclosion mass using a cubic model (Table 2). In contrast (Table 3), maximum mass reached prior to laying the 2nd egg pod (Max mass bf. pod 2) is best predicted (R^2 = 0.49) by clutch size pod 1, using an exponential function (Table 3). Fig. 2 provides visual comparisons of linear *vs* nonlinear models for two contrasts. In each case, the nonlinear models provided a slightly better fit, as expected based on R^2.

Table 3. Some pair-wise contrasts among female grasshopper reproductive traits, showing best-fit nonlinear models and resulting coefficients of determination (R^2) for each contrast. See Methods section for explanation of terms. "bf." refers to "before".

Independent variable	Dependent variable	Model	R^2
Max-mass bf. pod 1	Clutch-size pod 1	Poly-3	0.47
Max-mass bf. pod 1	Mass pod 1	Exponential	0.37
Max-mass bf. pod 1	Clutch-size pod 2	Poly-3	0.38
Mass pod 1	Clutch-size pod 1	Logarithmic	0.42
Mass pod 1	Max mass bf. pod 2	Poly-3	0.36
Mass pod 1	Clutch-size pod 2	Poly-3	0.38
Mass pod 1	Mass pod 2	Poly-3	0.31
Clutch-size pod 1	Mass pod 1	Poly-3	0.48
Clutch-size pod 1	Max mass bf. pod 2	Exponential	0.49
Clutch-size pod 1	Clutch-size pod 2	Poly-3	0.52
Clutch-size pod 1	Mass pod 2	Poly-3	0.22
Time: eclos.-pod 1	Time from pod 1 to 2	Poly-3	0.17
Max-mass bf. pod 2	Clutch-size pod 2	Poly-3	0.51
Max-mass bf. pod 2	Mass pod 2	Poly-3	0.59
Mass pod 2	Clutch-size pod 2	Power	0.49

Principal component analysis.— We classified the variables into two groups: morphological and fecundity variables. We then employed Principal Component Analysis (PCA) on each group to determine the minimal number of variables from each group that represent the highest portion of the variation. This allowed us to examine the relationship between the morphological (independent) and fecundity (dependent) variables by employing models with higher degrees of freedom. The resulting extraction communalities were all high, indicating they are good representations of the overall variation in the data. Further PCA extraction of the morphological data (Table 4) confirms that maximum mass prior to the 1st oviposition, head width, and pronotum length, explain 88.5% of the total variation of morphological variables. Note that max mass prior to the 1st oviposition (Pod 1) is highly correlated (0.94) with the first component, while weakly correlated (-0.142 and 0.039) with the other components (Table 4). Head width is highly correlated with the second component (0.83) while correlation for the pronotum is highest for the third component (0.51). On the other hand, we also note that femur length may be included in this list (perhaps in *lieu* of pronotum), since it shows a similar correlation structure

Table 4. PCA analysis of morphological variables. "bf." refers to "before".

	Component		
	1	2	3
Head width	0.556	0.828	-0.007
Pronotum length	0.802	-0.045	0.509
Femur length	0.835	-0.079	-0.421
Eclosion mass	0.877	-0.059	-0.143
Maxmass bf. pod 1	0.944	-0.142	0.039
Maxmass bf. pod 2	0.887	-0.195	0.040

	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	4.097	68.278	68.278
2	0.755	12.576	80.854
3	0.459	7.655	88.510
4	0.391	6.525	95.035
5	0.241	4.021	99.055
6	0.057	0.945	100.000

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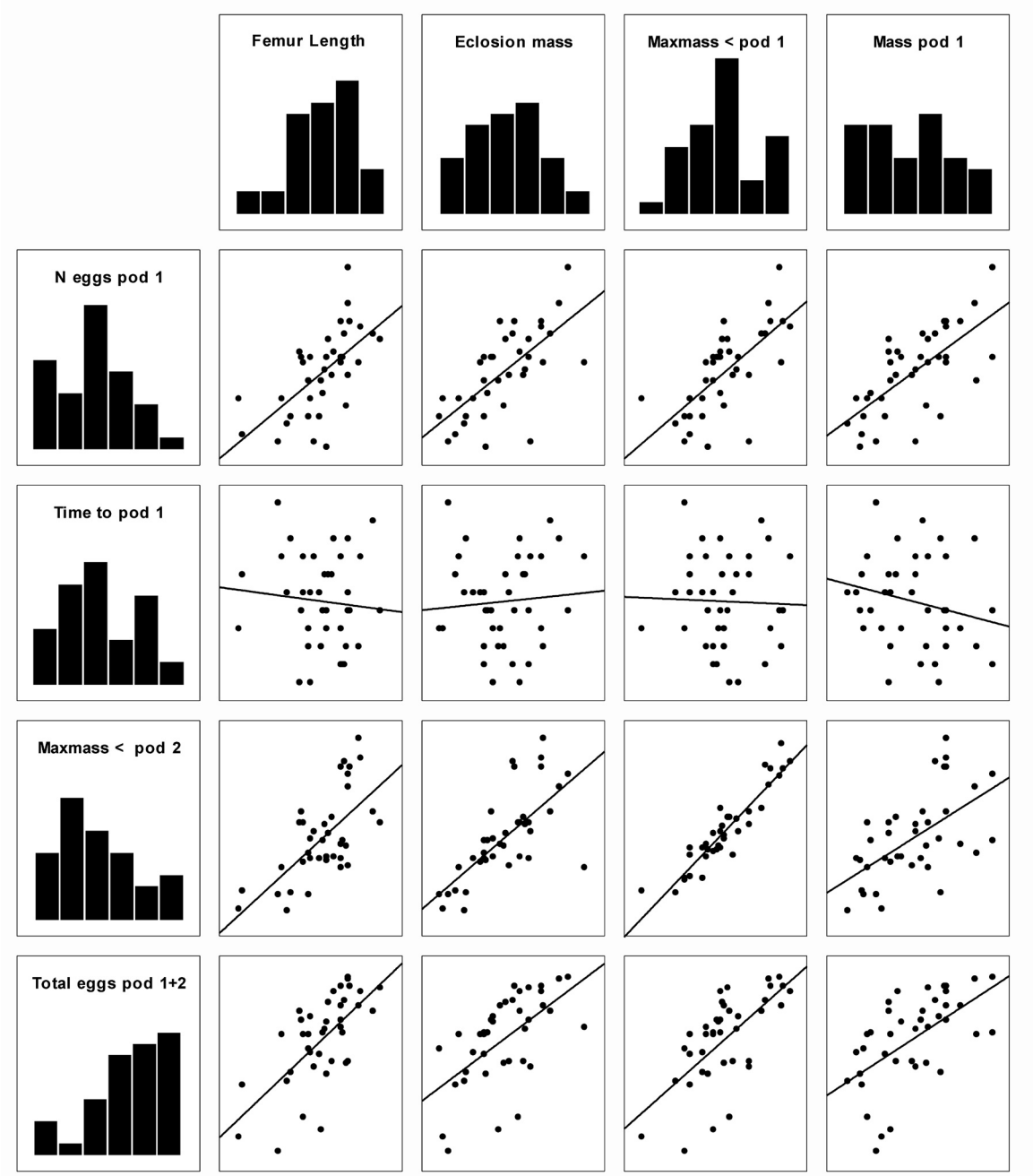

Fig. 1. Linear regression plots for selected female grasshopper morphological (top row) and fecundity (left column) variables. Consult Table 1 for correlation coefficients and significance, and Methods section for definition of terms.

with factor components as pronotum does.

We also constructed a dendrogram (Fig. 3) to examine the homogeneity between our variables in terms of their correlations. The dendrogram shows that max-mass prior to pod 1, max-mass prior to pod 2, head width, and eclosion mass are homogenous in terms of partial correlations, as represented by their shared fork. Next, pronotum length joins this group as another distinctive variable of the subset. Hence, femur, pronotum length and any one of the variables among eclosion mass, head width, max-mass prior to pod 1 and max-mass prior to pod 2, can be used for a suitable prediction. See Kachigan (1986) for technical details of this method.

We used the same approach as explained above for the fecundity variables (Table 5) and obtained the three variables that explain over 80% of the variation as clutch-size pod 1, time from eclosion to 2nd oviposition, and mass pod 1. We then predicted various dependent variables using the selected morphological and fecundity variables. Furthermore, we employed the best subset factorial regression using Mallow's C_p criterion, to allow the interactive effects of multiple continuous predictor variables to derive models that satisfy the best subset regression quality measures for a number of our dependent variables (Table 6).

Discussion

Our analyses demonstrate strong correlations among most morphological variables, and among some fecundity variables.

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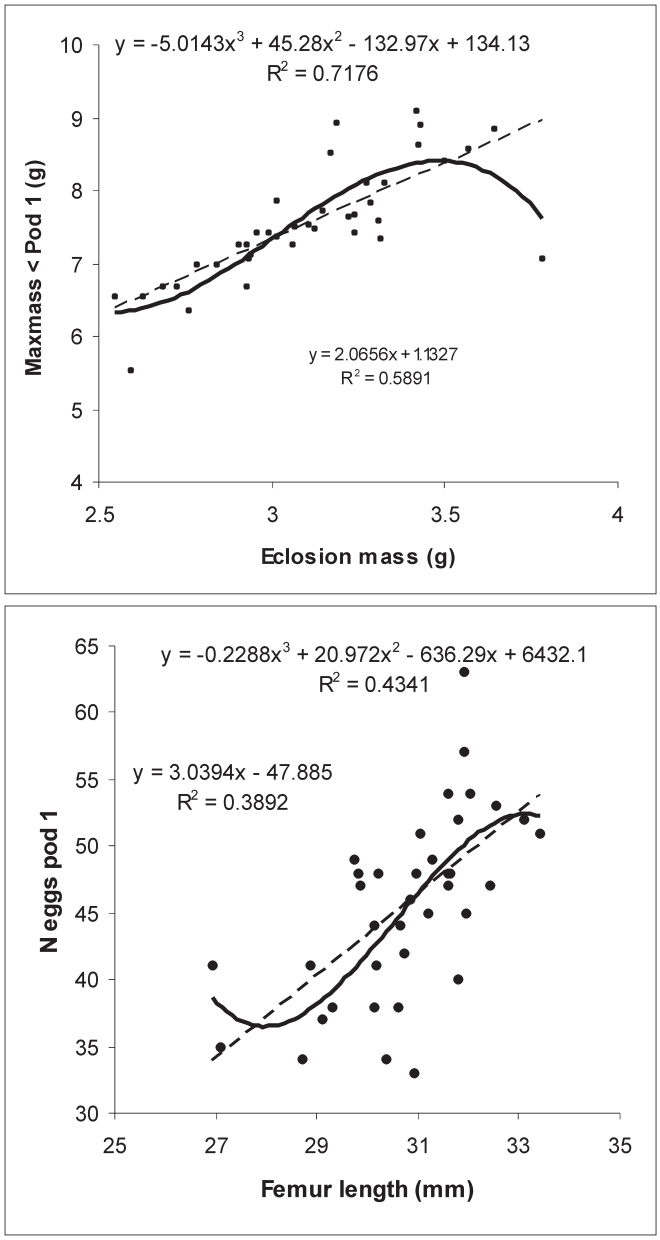

Fig. 2. Comparisons of linear *vs* nonlinear models of two bivariate morphological and reproductive relationships in female grasshoppers.

Our study also indicates that in female *R. microptera* grasshoppers, morphological variables can predict fecundity variables, except for time to oviposit. Finally, our data suggest that mating influences both time of oviposition and clutch size.

Our initial linear analysis of all pair-wise contrasts (Table 1) suggests that: 1) the morphological variables correlate well with one another, particularly femur length, which correlates positively with eclosion mass, maximum mass prior to pod 1, and maximum mass prior to pod 2 (all with $r \geq 0.66$). Also, maximum mass prior to pod 1 is highly positively correlated with maximum mass prior to pod 2 ($r = 0.93$). This suggests that size, mass, and volume/capacity are all highly correlated, a finding that is not surprising (Radtke *et al*. 2006). 2) Morphological variables (particularly femur length and maximum mass reached prior to oviposition, but not head width or pronotum length), correlated well with clutch size and

Table 5. PCA analysis of fecundity variables.

	Component		
	1	2	3
N eggs pod 1	0.883	0.013	-0.081
Time: eclose- pod 1	-0.154	0.788	0.400
N eggs pod 2	0.868	0.085	0.336
Mass pod 1	0.757	-0.114	-0.408
Mass pod 2	0.732	-0.190	0.268
Interval: pod 1 - pod 2	0.175	0.835	-0.371
Time: eclosion - pod 2	0.014	0.990	-0.032
Total eggs pod 1+2	0.881	0.114	0.023

Initial Eigenvalues			
Component	Total	% of Variance	Cumulative %
1	3.473	43.415	43.415
2	2.369	29.616	73.031
3	0.657	8.214	81.245
4	0.625	7.817	89.061
5	0.537	6.712	95.774
6	0.213	2.668	98.442
7	0.117	1.467	99.909
8	0.007	0.091	100.000

pod mass. 3) We found no correlation between morphological features and time to oviposit either 1st or 2nd egg pods. 4) When comparing among fecundity variables, clutch-size pod 1 was a strong predictor for clutch-size pod 2, and also for mass of both pods 1 and 2. Likewise, clutch-size pod 2 correlated well with mass pod 2. 5) Time to oviposit was generally unrelated to body size or mass, or clutch size or mass. Indeed, out of 27 pair-wise contrasts involving time *vs* morphology or fecundity variables, we found only one significant correlation: time from adult eclosion to pod 1 was negatively correlated with mass of pod 1 ($r = -0.32$), and *vice versa* (Table 1, Fig. 1). Previous studies on lubber grasshoppers have also demonstrated a relationship between body size or mass and clutch size, but not age of oviposition (Moehrlin & Juliano 1998, Luker *et al*. 2002, Hatle *et al*. 2002, but see Hatle *et al* 2004). 6) When examining the effects of mating, we noted that females that mated early tended to oviposit earlier than females that mated later, and that females with many sexual partners tended to lay smaller clutches (Table 1). In aggregate, these results suggest that clutch size and pod mass are strongly determined by body size and mass at eclosion, and that the mass or clutch size of the 1st egg pod is a good predictor for mass and clutch size of the 2nd egg pod. In contrast, the timing of oocyte development and timing of oviposition are independent of body size and mass at eclosion in this study. However, timing of oviposition appears to be related to timing of mating, while excessive mating can lower clutch size.

Among grasshopper species, clutch size, egg size, and timing of oviposition correlate moderately well with body size and mass: larger species tend to lay larger clutches of larger eggs at longer intervals (Bellinger & Pienkowski 1985, Stauffer & Whitman 1997). For example, Kriegbaum (1997) found that body size predicted both clutch size and time to lay the first pod, among seven species of grasshopper.

Within grasshopper species, size and mass variables are usually highly correlated (*e.g.*, Shotwell 1941; Norris 1950, 1952; Blackith & Verdier 1960; Farrow 1982; Atkinson & Begon 1988; Kosal & Niedzlek-Feaver 1997), but the situation for reproductive variables is less clear. Some authors have found that ovariole number, clutch size, egg size, or oocyte development rates are associated with body

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Table 6. Best-fit models to explain various dependent variables, determined using variables obtained through principal component analysis (see text). Note that the variables joined by "x" refer to derived interaction terms; they are not the product of *e.g.*, Head width multiplied by Femur length, but instead indicate their interaction term through PCA.

Mass pod 1 = -16.5 + (0.89 × Pronotum) + (0.084 × Head × Femur) – (0.0040 × Head × Pronotum × Femur)

Clutch-size pod 1 = 0.840 + (3.78 × Maxmass prior to pod 1) + (8.10 × Mass pod 1)

Mass pod 2 = -34.5 + (2.01 × Pronotum) + (0.146 × Head × Femur) – (0.0146 × Head × Pronotum × Femur) – (0.00802 × Head × Pronotum × Femur)

Time from pod 1 to 2 = 190 – (9.61 × Pronotum) – (8.24 × 10^7 × Head × Femur) + (0.0458 × Head × Pronotum x Femur)

Max-mass prior to pod 2 = -0.236 + (0.860 × Maxmass prior to pod 1) + (0.652 × Mass pod 2)

Total eggs laid (pods 1 + 2) = -209 + (9.63 × Femur)

size (Norris 1950, 1952; Richards & Waloff 1954; Blackith & Blackith 1968; Farrow 1975, 1982; White & Contreras 1979; Hugueny & Louveaux 1986; Atkinson & Begon 1987, 1988; Butlin et al. 1987; Ritchie *et al*. 1987; Wall & Begon 1987; Landa 1992; Moehrlin & Juliano 1998; Cueva del Castillo *et a*l. 1999; Hatle *et al*. 2002; Danner & Joern 2004), whereas others have not (Smith 1972, Dearn 1977, Atkinson & Begon 1987, Butlin *et al*. 1987, Ritchie *et al*. 1987, Luker *et al*. 2002). In lubber grasshoppers, egg size appears to be unresponsive to dramatic diet-induced changes in body mass and size in females (Moehrlin & Juliano 1998, Hatle *et al.* 2002).

Drawing generalities from among these intraspecific studies is difficult because of the great diversity of confounding factors. For example, some studies compared different populations surviving in different latitudes, altitudes, or plant communities. Others explored body size and fecundity as a consequence of different nutrition, crowding, phase state, season, age, temperature, photoperiod, wing length, mating status, or predation risk. In some grasshoppers, fecundity is influenced by number of matings (Walker *et al*. 1999), and in others, larger individuals mate more (Cueva del Castillo & Núñez-Farfán 2002, Cueva del Castillo 2003), or females prefer large males (Kosal & Niedzlek-Feaver 1997). An additional confounding factor is that time to lay in some species is influenced by environment-induced reproductive diapause (see Uvarov 1977, Weissman & French, 1980, Weissman 1979, Lightfoot & Weissman 1991) or social factors (Stauffer & Whitman 1997, Stauffer *et al.* 1998). To begin to understand intraspecific variation in fecundity as it relates to body size and mass, it is probably best to start with one population, with individuals raised under identical and optimal conditions.

Our finding that time to lay is unrelated to body size and mass, relates to reproductive allocation. Assuming that large females assimilate more nutrients than small females (Belovsky 1986, Peters 1989), then large females can allocate this added nutrition to affect number, timing, or quality of offspring. Apparently *R. microptera* chooses number, as suggested by the highly significant positive correlations of body mass and size with clutch size, and the lack of correlation with either time to reproduce (Table 1) or egg quality, as measured by egg mass. In contrast, oocyte development rate, body size, and clutch size respond to nutrition, but not photoperiod (Luker *et al.* 2002, Hatle *et al*. 2004). In other words, for adults only, time to lay appears to vary with nutrition, but not body mass or photoperiod in this species. However, our present study examined adults given surplus food. If, instead, we had raised nymphs under poor nutrition, we would expect a correlation between adult body mass and time to lay.

As previously mentioned, social factors also affect grasshopper ovipositon. Oocyte development rates and oviposition timing in some grasshoppers, including *R. microptera*, are influenced by male presence, mating, and social grouping (see review in Walker *et al.* 1999). The data from our present paper derived from an experiment in which these factors varied. Perhaps the effects of these factors masked our ability to detect relationships between time and morphological or fecundity factors. Still, in our study, clutch size for Pod 2 was significantly negatively correlated with the number of matings, and, the time to lay Pod 2 correlated with age of 1st mating (Table 1). The large number of negative correlations of mating x other variables (Table 1), suggests that, aside from providing fertility, excessive mating may harm females. Indeed, literature on grasshoppers and other insects suggests a negative effect of excessive mating (Parker 1979, Thornhill & Alcock 1983, Rowe 1994, Clutton-Brock & Langley 1997, Reinhardt & Köhler 1999, Walker

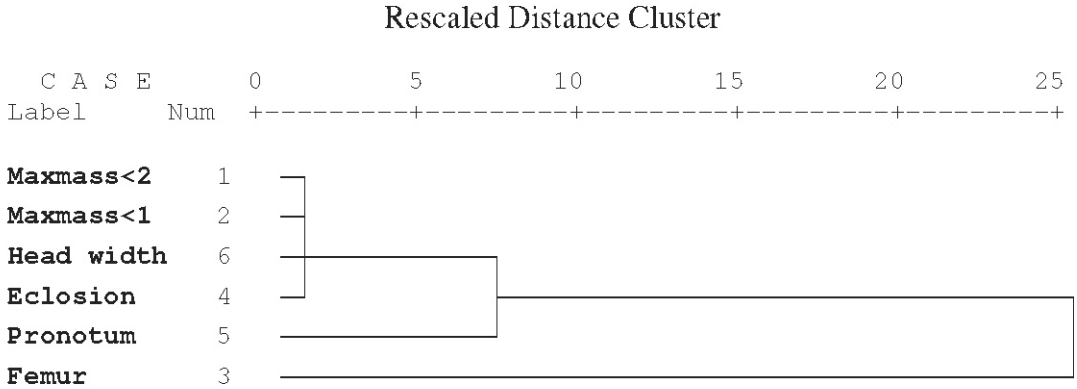

Fig. 3. Dendrogram showing the intercorrelative structure of morphological variables.

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et al. 1999). Pitnick and García-González (2002) noted that in *Drosophila*, harm to mated females increased with male body size.

In our study, nonlinear models produced fits with higher R^2 than linear models. This was not surprising. However, we sought to derive the best predictors for our fecundity variables, and therefore, conducted principal component analysis, along with statistical clustering. This technique provided us with the best subset of predictors, with the least amount of information loss for further modeling. Moreover, we also obtained the best subset of the fecundity characteristics to be predicted (dependent variables), thus reducing the number of models extracted from the same dataset. Hence, not all morphological characteristics (independent variables) need to be measured to predict fecundity.

Finally, we employed multiple regressions with stepwise selection to generate best predictors and then compared these with those produced using PCA. In nearly all cases, the regression-selection came to the same conclusion as the PCA. For example, regression indicated that maximum body mass prior to Pod 2 was an important fecundity variable, as did PCA. The results further confirm the usefulness of PCA, in that the best regression model was a function of only two predictors, with minimal loss of variation.

Caveat.—In this paper, we made a large number of contrasts without adjusting significance levels for multiple comparisons. Hence, it must be assumed that a certain number of our "significant" outcomes do not represent true biological relationships. Also, we derived our raw data from a previously published experiment (Walker *et al*. 1999), in which female grasshoppers experienced different levels of social grouping and mating. These added variables may have clouded our ability to detect significant correlations. Finally, in the original study, ~ 8% of extremely large or small animals were excluded from the experiment, so as to have more homogenous groups; elimination of such outliers may have reduced our ability to detect significant size/mass correlations in our present study.

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