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ARTICLE

Fluctuating asymmetry in an extreme morphological adaptation in the Chilean bee *Xeromelissa rozeni* (Hymenoptera: Colletidae)

Margarita Miklasevskaja and Laurence Packer

Abstract: Fluctuating asymmetry (FA), random deviations from perfect symmetry in bilateral traits, is a common inverse measure of developmental stability (DS), which is related to one's ability to buffer against environmental and genetic perturbations. There is a widespread hypothesis that heterozygosity grants an increased ability to compensate for developmental errors caused by genetic and environmental factors, rendering homozygous individuals less symmetric than heterozygous ones. In addition, if natural selection on FA is common, nonessential traits should exhibit higher asymmetry than functionally essential traits. This is especially well tested in haplodiploid organisms, which present a clear distinction between "homo"zygosity (males) and heterozygosity (females). Relatively few FA studies looked at this relationship in hymenopterans or in haplodiploid organisms in general and the results are rather inconsistent. This study compares FA measurements of seven parts of the maxillary palpus, with sclerotized and membranous parts scored separately, and two wing venation characters for males and females of the Chilean bee *Xeromelissa rozeni* (Toro and Moldenke, 1979). The results of this study suggest that there is an equally strong selection force for maxillary palp symmetry in both males and females leading to a relatively low FA in both sexes, and that less functional traits exhibit higher FA due to relaxation of selection. Lastly, we stress the importance of testing a larger number of independent traits.

Key words: developmental instability, haplodiploidy, maxillary palpomeres, essential structures, new species, morphology, *Xeromelissa rozeni*, colletid bees.

Résumé : L'asymétrie fluctuante (AF), soit des écarts aléatoires par rapport à la symétrie parfaite des caractères bilatéraux, est une mesure inverse répandue de la stabilité du développement (SD), qui est reliée à la capacité d'atténuer les effets de perturbations environnementales et génétiques. Une hypothèse répandue veut que l'hétérozygotie confère une capacité accrue d'atténuer les erreurs de développement causées par des facteurs génétiques et environnementaux, rendant les individus homozygotes moins symétriques que les hétérozygotes. En outre, si la sélection naturelle pour l'AF est répandue, les caractères non essentiels devraient présenter une plus grande asymétrie que les caractères essentiels sur le plan fonctionnel. Cela est particulièrement bien établi pour les organismes haplodiploïdes, caractérisés par une distinction claire entre l'« homo »zygotie (mâles) et l'hétérozygotie (femelles). Assez peu d'études de l'AF se sont penchées sur ce lien chez les hyménoptères ou chez les organismes haplodiploïdes en général, et les résultats ne sont pas très cohérents. La présente étude compare des mesures de l'AF de sept parties du palpe maxillaire, les parties sclérotisées et membraneuses étant notées séparément, et deux caractères de la nervation alaire pour des abeilles chiliennes *Xeromelissa rozeni* (Toro et Moldenke, 1979) mâles et femelles. Les résultats donnent à penser que la force de sélection pour la symétrie des palpes maxillaires est similaire pour les mâles et les femelles, se traduisant par une AF relativement faible pour les deux sexes, et que les caractères moins fonctionnels présentent une AF plus grande en raison du relâchement de la sélection. Enfin, nous insistons sur l'importance de vérifier un grand nombre de caractères indépendants. [Traduit par la Rédaction]

Mots-clés : instabilité du développement, haplodiploïdie, palpomères maxillaires, structures essentielles, nouvelle espèce, morphologie, *Xeromelissa rozeni*, colletidés.

Introduction

Fluctuating asymmetry (FA), random deviations from perfect symmetry in bilateral traits, is one of three types of recognized asymmetry (Palmer and Strobeck 1986). The others are directional asymmetry (DA) and antisymmetry. These deviations from symmetry are differentiated based on distributions of the mean and variance of uncorrelated differences between sides of paired traits (Palmer and Strobeck 1986; Graham et al. 1993, 2010; Carter et al. 2009). There are three main distribution patterns of asymmetry: a normal distribution of right side minus left side differences, which is suggestive of FA; a bimodal distribution, which reflects antisymmetry (e.g., claws of male fiddler crabs (genus *Uca* Leach, 1814); Pratt and Mclain 2002), and a normal distribution about a mean other than zero that signifies DA (e.g., the skulls of modern toothed whales (suborder Odontoceti); Cranford 1992). FA is a common inverse measure of developmental stability (DS), which is defined as the ability to buffer development against environmental and genetic perturbations (Palmer 1994).

Lerner (1954) was the first to hypothesize why heterozygous individuals have added buffering capacity. He proposed that greater heterozygosity grants an increased ability to compensate for morphometric variation caused by genetic and environmental factors,

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M. Miklasevskaja and L. Packer. Department of Biology, York University, 4700 Keele Street, Toronto, ON M3J 1P3, Canada.
Corresponding author: Margarita Miklasevskaja (e-mail: margmik@gmail.com).

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such that individual phenotypes can be expressed more precisely, i.e., show less FA (Hall 2005). Conversely, more homozygous individuals will have lower capacity to buffer against perturbations, thus developing more FA. Although this claim has been repeated many times (e.g., Leary et al. 1983, 1984, 1985*a*, 1985*b*; Clarke et al. 1992; Palmer and Strobeck 2003), some studies show no such association (e.g., Smith 1982; Hosken et al. 2000). However, in addition to heterozygosity, there are two other hypotheses on the determinants of FA: ploidy effects and genomic coadaptation. Ploidy influences FA not only through potential heterozygosity but also via influences of gene dosage (Leary et al. 1985*b*). The latter arises because individuals with more allele copies, even if all are identical, are better adapted to deal with perturbations (Leary et al. 1985*b*; Clarke 1997). Genomic coadaptation affects FA when pre-established genic interactions between chromosome homologs are disturbed, as often occurs in hybrids that often show higher levels of FA. Neither of these models alone is able to explain consistently the contradicting results of all FA studies (Allendorf and Leary 1986; Woolf and Markow 2003; Graham et al. 2010).

Multiple studies have tested for negative correlation between heterozygosity and FA by comparing levels of asymmetry and heterozygosity among individuals of the same population. Some found a negative correlation (e.g., Leary et al. 1983, 1985*a*; Clarke et al. 1992), whereas others found a positive one or no correlation at all (e.g., Smith 1982; Clarke et al. 1986; Hosken et al. 2000).

To date, most studies of FA have been performed on diploid organisms. The problem with diploid systems occurs when attempting to distinguish between heterozygosity and gene coadaptation; because they are not independent factors, it is nearly impossible to manipulate one without unintentionally affecting the other (Allendorf and Leary 1986). In contrast to diploid systems, haplodiploid animals such as all Hymenoptera and Thysanoptera, as well as some Homoptera, Coleoptera, and Acari (Borgia 1980; Cruickshank and Thomas 1999), provide a convenient solution to this problem. In a haplodiploid genetic system, females are produced from fertilized eggs, while healthy males are produced from unfertilized eggs. In most haplodiploids, sex is determined not by ploidy per se, but by a single sex-determining locus (van Wilgenburg et al. 2006; Huang et al. 2014) in a system called single locus complementary sex determination (sl-CSD). Heterozygosity at this locus results in a female, whereas homozygosity (diploid) or hemizygosity (haploid) results in a male. As a result of this system, females are heterozygous for at least some loci, whereas hemizygous males will have no heterozygosity at all (Zayed 2004). Occasionally, there is homozygosity at the sex locus resulting in a diploid male that is inviable, infertile, or produces triploid offspring (Zayed and Packer 2005; Huang et al. 2014). In studies of FA in haplodiploids, diploid males will weaken any differences between the sexes by displaying female levels of asymmetry and heterozygosity. However, in species with sexual size dimorphism, diploid males can often be detected by their larger body size and (or) mass (Owen and Packer 1994). Thus, haplodiploid systems not only provide a natural partition between heterozygosity (females) and hemizygosity (males), but also serve as a convenient model to differentiate the effects of ploidy, heterozygosity (Owen 2012), and gene coadaptation on FA and DS (Clarke et al. 1992).

Relatively few FA studies have been performed on hymenopterans or other haplodiploids, and the results are rather inconsistent among taxa. Bruckner (1976), for example, looked at morphometric wing asymmetry between drones and females of the western domesticated honeybee (*Apis mellifera* L., 1758) and found that drones were consistently more asymmetrical than females. Clarke et al. (1992) observed a similar pattern in the same species, but suggested that genetic coadaptation was the main cause of higher FA in males. Another study on *A. mellifera* (Smith et al. 1997) also showed higher asymmetry in males compared with females, but the authors concluded that this difference was in the form of DA. More taxa were studied by Clarke et al. (1986) who used a combination of morphometrics and meristic measurements on five species from four hymenopteran families, as well as two species from a single family of Thysanoptera, and concluded no significant difference in asymmetry between males and females for any of the 60 comparisons. Studies of *Oncothrips tepperi* (Karny, 1911) also found no significant difference between males and females (Crespi and Vanderkist 1997). Lastly, there was no difference in levels of FA between two forewing characters between males and females of a solitary carpenter bee, *Xylocopa appendiculata circumvolans* (Smith, 1873) (Kudo and Mori 2000).

Following Palmer's (1994) protocol for selecting a trait, most studies have focused on a single trait—a wing, specifically its venation, which, as he suggested, likely has low plasticity and low modification due to wear. However, since insect wings can be fully functional with up to a 12% asymmetry or even compensated for biomechanically above this 12% threshold (Fernández et al. 2012), developmental stability may not be as crucial as initially thought.

Here we consider FA in a number of traits for the colletid bee *Xeromelissa rozeni* (Toro and Moldenke, 1979) using both wing and mouthpart characters. *Xeromelissa rozeni* is a member of a species group with enormously elongate maxillary palpi, presumably to permit extraction of nectar from the deep nectaries of their floral hosts (seemingly all species of the Chilean bell flower, genus *Nolana* L.f.; Solanaceae) (e.g., Rozen 2003). Deep nectaries are, in turn, likely an adaptation to prevent evaporation of nectar in the extremely arid environment within which these species of *Xeromelissa* are found.

The maxillary palpi are composed of six sclerotized palpomeres with five membranous regions connecting adjacent palpomeres. The first two and the last two palpomeres are relatively short, whereas palpomeres 3 and 4 are extremely long, with a mean of 1.74 mm (SD = 0.10, N = 98) and a mean of 1.62 mm (SD = 0.11, N = 98) in length, respectively (Fig. 1A). These two palpomeres are laterally flattened and slightly concave on the medial surface. These structures presumably work by aligning the concave inner margins to form a tube that likely draws up the nectar by capillary action, aided by lapping motions (Krenn et al. 2005). Rozen and Wyman (2015) also concluded this from their observations of *X. rozeni* mouthparts. Like other colletid bees, *Xeromelissa* females provide an unusually high ratio of nectar to pollen for their offspring in a provision mass that is in a brood cell lined with a waterproof cellophane-like material (Cane 1983; Rozen and Wyman 2015). Hence, it is probably essential for the maxillary palpi to be symmetrical to function properly, and this may be especially important for females that do all of the provisioning for the offspring. Consequently, there probably is strong selection acting against asymmetry of the mouthparts, especially those of females, especially of the extremely elongate palpomeres 3 and 4.

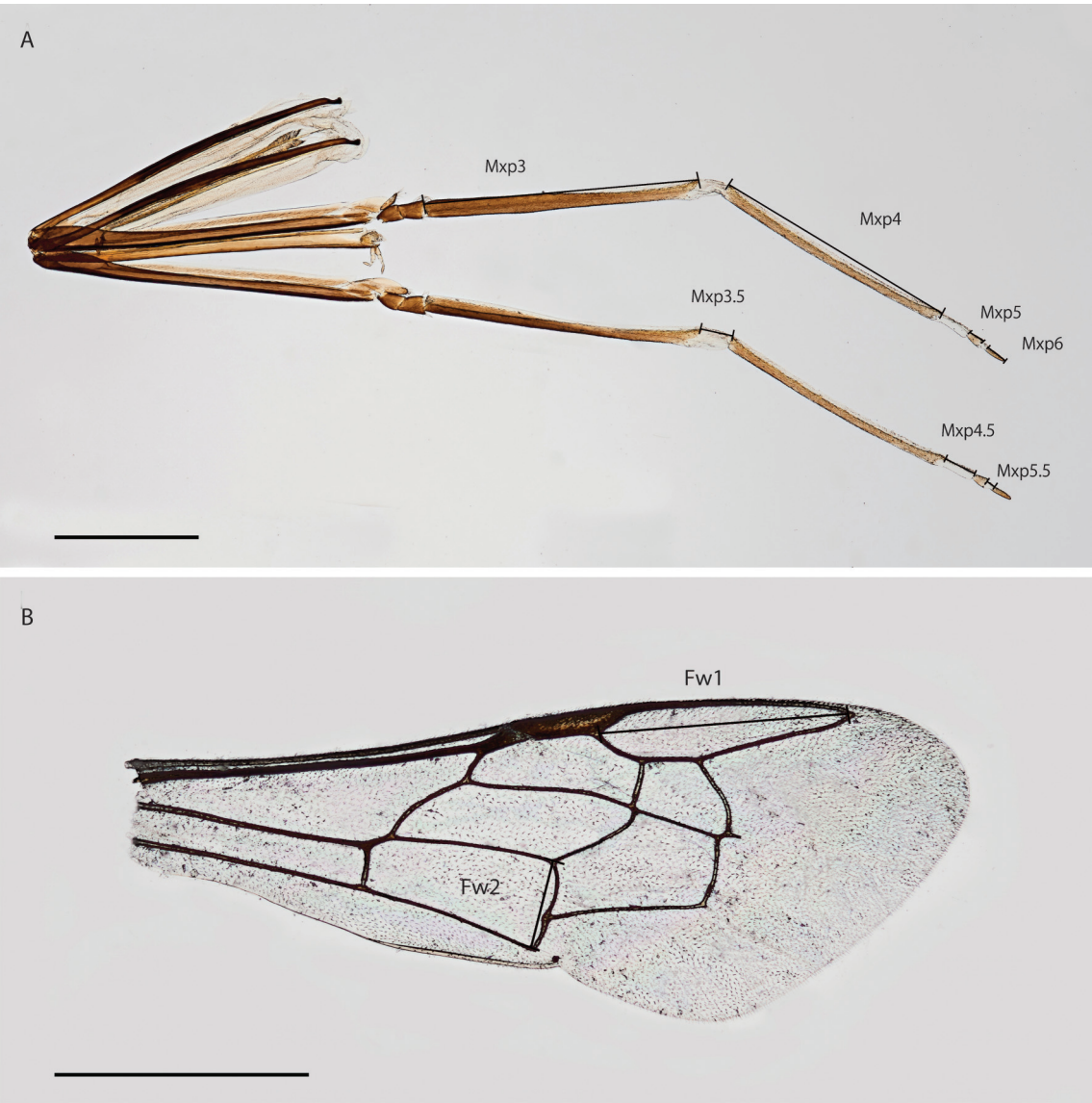
In this paper, we look at FA differences between males and females of *X. rozeni*, focusing on the sclerotized parts of palpomeres 3–6 and the three membranous parts between them. In addition, two wing-venation measurements, defined by four landmarks (Fig. 1B), are subjected to the same analyses. The following hypotheses are tested. First, that FA is greater in hemizygous males than in diploid females. Second, that membranous parts of the maxillary palpi show higher FA than sclerotized ones; this is expected because they do not directly participate in feeding-tube capillary action. Third, that palpomeres 3 and 4 have reduced FA compared with the other palpomeres and the wing-measurement variables, because of their seemingly great importance in nectar uptake.

Materials and methods

Collection and measurements

Xeromelissa rozeni was collected from east of Chañaral in Region III in Chile, in October 2011, using pan traps containing propylene glycol. Voucher specimens are housed at the Packer Collection at York University. Fifty males and 49 females were collected and stored in 100% ethanol until processing for measurement. Intact

Fig. 1. Images of (A) maxillary palpi and (B) forewing of the Chilean bee *Xeromelissa rozeni* to show the landmarks used for measurements. Scale bar is 1 mm in length. Figure appears in colour on the Web.



maxillary palpi were detached from the rest of the maxilla at the base of palpomere 1 and both forewings were also removed for measurement. To visualize distinct landmarks of the palpomeres, dissected mouthparts were cleared in a 10% solution of KOH for 4 h. Forewings and cleared maxillary palpi were mounted on slides with glycerine and imaged using a Visionary Digital BK Plus imaging system using a Canon EOS40D digital SLR camera with transmitted light. Measurements were taken using Adobe Photoshop© version CS6 to an accuracy of 0.1 μm. The lengths of the ventral surface of both sclerotized and membranous parts of palpomeres 3–6 were measured for all individuals. Each of those palpomeres, except the last, consists of both a sclerotized basal region followed by a membranous portion. Thus, individual traits are indicated as follows: the sclerotized part of palpomere 3 is designated Mxp3 and its membranous portion (between Mxp3 and Mxp4) is designated Mxp3.5; the fourth and fifth palpomeres were similarly designated (Fig. 1A). MxpTotal denotes a sum of Mxp3 to Mxp6, including the membranous portions. Marginal cell length and apical width of the 2nd cubital cell were also measured for 49 females and 50 males. The lengths of these two traits are denoted as Fw1 and Fw2, respectively (Fig. 1B).

To ensure that measurements were uninfluenced by any potential unconscious bias, the palpi and wings were arranged by an assistant so that the measurements were taken by the senior author who was blind to the sex, side, and specimen being measured. Following Palmer (1994), each trait was measured twice on a different day without reference to the previous measurements to obtain estimates of measurement error.

Analysis

The entire analysis was completed using Palmer and Strobeck's (2003) protocols; this paper is used for all of the methodological details noted below except where stated otherwise.

Before proceeding with FA analysis, the data were inspected for errors in raw measurements, as well as aberrant individuals that may artificially inflate FA. A scatterplot of the difference between repeated measurements, as well as the difference in right side minus left side (R – L) of paired traits, was used to visualize apparent outliers. Outlier significance was tested using Grubb's test (Rohlf and Sokal 1995) following a sequential Bonferroni *p*-value correction for multiple tests.

Table 1. Mean (±SE) trait size, directional asymmetry (DA), kurtosis, and skew for all traits and both sexes of the Chilean bee *Xeromelissa rozeni*.

				R – L			
Trait	Sex	*N*	(R + L)/2, mean ± SE	Mean ± SE	Kurtosis	Skew	Spearman's ρ*
Mxp3	Male	50	1.732±0.014†	−0.0014±0.004	1.881	0.130	0.026
	Female	48	1.758±0.014†	0.0047±0.005	2.265†	0.409	−0.074
	Both	98	1.745±0.010†	0.0016±0.003	1.939†	0.274	2.490×10^{-4}
Mxp3.5	Male	50	0.157±0.003	−0.0022±0.004	0.879	0.016	0.116
	Female	48	0.157±0.003	0.0022±0.004	−0.673	0.223	−0.124
	Both	98	0.157±0.002	−0.0001±0.003	0.0001	0.141	0.017
Mxp4	Male	50	1.585±0.016†	−0.0048±0.004	6.462†	1.495†	−0.041
	Female	48	1.667±0.016†	−0.0069±0.004	1.167	−0.859	−0.071
	Both	98	1.626±0.011†	−0.0058±0.003	4.005†	0.417	−0.054
Mxp4.5	Male	50	0.181±0.003†	−0.0014±0.002	0.980	−0.202	−0.195
	Female	47	0.196±0.003†	−0.0023±0.002	0.574	−0.736	−0.185
	Both	97	0.189±0.002†	−0.0018±0.002	0.725	−0.519	−0.137
Mxp5	Male	50	0.092±0.001†	−0.0014±0.001	3.436†	0.231	0.041
	Female	47	0.099±0.001†	−0.0033±0.002	9.219†	−2.055†	−0.147
	Both	97	0.096±0.001†	−0.0023±0.001	6.638†	−0.997†	−0.085
Mxp5.5	Male	50	0.043±0.001†	0.0017±0.001	−0.298	0.505	−0.076
	Female	47	0.051±0.001†	−0.0013±0.001	0.767	−0.834	−0.160
	Both	97	0.047±0.001†	0.0002±0.001	0.543	0.289	−0.064
Mxp6	Male	50	0.135±0.001	0.0008±0.001	0.322	−0.600	0.032
	Female	47	0.136±0.001	0.0014±0.001	0.101	−0.372	−0.207
	Both	97	0.136±0.001	0.0011±0.001	0.403	−0.505	−0.077
MxpTotal	Male	47	3.91±0.035†	−0.0150±0.008	0.063	−0.120	0.210
	Female	44	4.068±0.028†	−0.0098±0.010	7.552†	−1.960†	0.330
	Both	91	3.986±0.024†	−0.0125±0.006	5.088†	−1.284†	0.021
Fw1	Male	52	0.994±0.008†	0.0079±0.007	35.324†	5.650†	0.096
	Female	49	1.064±0.008†	−0.0030±0.002	4.003†	−0.676	−0.019
	Both	101	1.029±0.007†	0.0023±0.004	55.472†	6.549†	0.209
Fw2	Male	49	0.343±0.003†	−0.0043±0.002	−0.056	−0.926	−0.113
	Female	50	0.372±0.003†	0.0024±0.003	0.922	−0.178	−0.19
	Both	99	0.357±0.003†	−0.0011±0.002	0.683	−0.363	−0.039

Note: For trait definitions refer to Figs. 1A, 1B.
*Spearman's coefficient of rank correlation between |R – L| and (R + L)/2, where R is right side and L is left side.
†Significant after Bonferroni correction.

A two-way, mixed-model ANOVA was set-up to test significance of the following three components: (1) nondirectional asymmetry (FA, if antisymmetry is absent) relative to measurement error; (2) directional asymmetry; (3) variation due to overall trait size among individuals (Palmer 1994, 1996; Palmer and Strobeck 2003)

Because there was a notable size difference among traits, unscaled indices, with and without measurement error (ME), were calculated to help visualize asymmetry variation among traits and sex.

A Levene's test of homogeneity of variances was used to assess the significance of |M2 – M1| (second measurement minus first measurement) differences among groups. Allometric variation was assessed from linear regressions of trait asymmetry |R – L| vs. trait size [(R + L)/2]. To test departures from normality, frequency distributions were examined visually followed by computation of skew and kurtosis. Asymmetries due to DA were examined using one-sample Student's *t* tests comparing the mean (R – L) difference to zero followed by a sequential Bonferroni *p*-value correction for multiple tests. Finally, to test whether FA varies among sex and traits, a two-way ANOVA and Levene's tests were computed for a combination of traits and sexes. Additionally, one-way ANOVAs were computed for males and females for each trait separately, as well as among the traits for each sex separately. All statistical analyses were computed using IBM SPSS Statistics for Windows© version 22.0.

Results

Descriptive data and tests of departures from ideal FA

Inspection of scatterplots revealed four extreme measurement errors that were found to be significant outliers. Therefore, data for two males and two females were excluded from all further analyses.

One-way ANOVA (sex) on trait size, (R + L)/2 (Table 1, column 4) for maxillary palp and forewing variables revealed that females were significantly larger than males for five out of seven palp traits and both forewing traits (all $P < 0.01$); the exceptions were Mxp3.5 and Mxp6.

DA, as mean (R – L), was not significant for any of the traits (Table 1, column 6). After sequential Bonferroni correction, Mxp3, Mxp4, Mxp5, and Fw1 exhibited significant leptokurtosis in both males and females; Mxp4, Mxp5, and Fw1 exhibited significant skew in both sexes (Table 1, columns 6 and 7, respectively). Cases of platykurtosis did not approach statistical significance, so antisymmetry was not evident.

The difference between sides (R – L) did not depend on trait size (R + L)/2 for any trait for either sex (Spearman's rank correlation coefficient (ρ), $P > 0.094$ for all groups; Table 1, column 8).

Descriptors of FA, ME, and tests of significance of FA relative to ME

Although female Mxp5 exhibited significant DA in a sides × individuals ANOVA (Palmer, 1994), this was not significant after sequential Bonferroni correction (Table 2*a*, column 1*a*). Trait-size variation among individuals was highly significant for all traits (Table 2*a*, columns 2 and 3). Between-side variation (FA) was significant relative to ME for all traits ($P < 0.001$; Table 2*a*, columns 5 and 6). Measurement error varied significantly among traits but not between sexes ($P < 0.001$ and $P = 0.49$, respectively, from a sex × trait ANOVA on |M1 – M2|; results not shown). Measurement error

Table 2. Results from a two-factor, mixed-model ANOVA on untransformed repeat measurements of maxillary palpi and wings of the Chilean bee *Xeromelissa rozeni*.

(*a*) Results from a two-factor, mixed-model ANOVA.

		Sides (S)		Individuals (I)			S × I interaction (test if asymmetries > ME)			Error (variance in repeats)	
Trait	Sex	MS_S (col. 1*a*)	df (col. 1*b*)	MS_I (col. 2)	*P* (col. 3)	df (col. 4)	$MS_{S\times I}$ (col. 5)	*P* (col. 6)	df (col. 7)	MS_{err} (col. 8)	df (col. 9)
Mxp3	Male	1.0×10^{-4}	1	0.043	4.49×10^{-22}	49	0.002	3.27×10^{-9}	49	4.0×10^{-4}	100
	Female	1.1×10^{-3}	1	0.028	3.69×10^{-22}	47	0.001	7.91×10^{-34}	47	5.0×10^{-5}	96
Mxp3.5	Male	4.0×10^{-4}	1	0.002	6.00×10^{-4}	49	0.001	7.17×10^{-7}	49	3.0×10^{-4}	100
	Female	2.0×10^{-4}	1	0.002	6.7×10^{-3}	47	0.001	3.39×10^{-15}	47	1.0×10^{-4}	96
Mxp4	Male	1.8×10^{-3}	1	0.064	2.84×10^{-32}	49	0.001	2.25×10^{-14}	49	2.0×10^{-4}	100
	Female	2.3×10^{-3}	1	0.032	2.39×10^{-25}	47	0.001	3.54×10^{-33}	47	4.2×10^{-5}	96
Mxp4.5	Male	1.0×10^{-4}	1	0.002	8.01×10^{-9}	49	0.000	3.92×10^{-11}	49	5.9×10^{-5}	100
	Female	2.0×10^{-4}	1	0.001	3.00×10^{-4}	47	0.000	1.30×10^{-14}	47	5.6×10^{-5}	96
Mxp5	Male	9.6×10^{-5}	1	0.000	4.31×10^{-5}	49	0.000	6.99×10^{-27}	49	7.4×10^{-6}	100
	Female	5.0×10^{-4}	1	0.000	3.00×10^{-4}	47	0.000	1.21×10^{-12}	46	2.1×10^{-5}	95
Mxp5.5	Male	1.0×10^{-4}	1	0.000	5.4×10^{-3}	49	0.000	6.58×10^{-7}	48	3.1×10^{-5}	99
	Female	8.2×10^{-5}	1	0.000	5.00×10^{-4}	47	0.000	1.00×10^{-4}	45	2.2×10^{-5}	94
Mxp6	Male	3.3×10^{-5}	1	0.000	1.36×10^{-9}	49	0.000	4.28×10^{-8}	46	9.6×10^{-5}	97
	Female	5.9×10^{-5}	1	0.000	1.62×10^{-5}	47	0.000	6.07×10^{-6}	42	1.7×10^{-5}	91
MxpTotal	Male	1.1×10^{-2}	1	0.227	1.00×10^{-4}	49	0.003	1.50×10^{-4}	46	1.0×10^{-3}	97
	Female	4.0×10^{-3}	1	0.131	1.04×10^{-4}	47	0.005	3.31×10^{-8}	43	3.3×10^{-4}	91
Fw1	Male	3.2×10^{-4}	1	0.032	1.14×10^{-15}	49	0.001	7.47×10^{-81}	49	3.2×10^{-6}	100
	Female	2.7×10^{-4}	1	0.012	1.87×10^{-26}	47	0.000	1.04×10^{-70}	43	2.3×10^{-6}	93
Fw2	Male	1.0×10^{-3}	1	0.002	0.33	49	0.000	3.02×10^{-119}	43	9.4×10^{-6}	90
	Female	2.2×10^{-4}	1	0.001	4.47×10^{-6}	47	0.0003	1.04×10^{-44}	43	6.5×10^{-6}	86

(*b*) Descriptors of FA and ME derived from the ANOVA results.

			FA10b (unscaled)				FA1 (mm)‡	
Trait	Sex	FA10a (mm; col. 10)*	Col. 11	df (col. 12)	Repeatability (ME5; col. 15)	FA4a (mm; col. 16)†	Mean (col. 17)	SE (col. 18)
Mxp3	Male	0.021	0.006	28.942	0.636	0.034	0.024	0.003
	Female	0.017	0.006	42.340	0.904	0.025	0.024	0.003
Mxp3.5	Male	0.014	0.062	20.653	0.500	0.024	0.022	0.003
	Female	0.017	0.067	37.885	0.818	0.025	0.025	0.003
Mxp4	Male	0.016	0.006	30.757	0.667	0.025	0.023	0.003
	Female	0.016	0.006	42.113	0.900	0.023	0.023	0.003
Mxp4.5	Male	0.009	0.032	31.158	0.673	0.014	0.012	0.002
	Female	0.010	0.033	34.464	0.755	0.016	0.014	0.002
Mxp5	Male	0.005	0.034	41.764	0.860	0.008	0.007	0.001
	Female	0.005	0.032	28.892	0.656	0.008	0.008	0.001
Mxp5.5	Male	0.005	0.076	21.843	0.520	0.008	0.009	0.001
	Female	0.003	0.036	15.068	0.418	0.006	0.007	0.001
Mxp6	Male	0.004	0.023	24.867	0.570	0.006	0.014	0.004
	Female	0.003	0.023	19.782	0.500	0.006	0.017	0.006
MxpTotal	Male	0.036	0.010	20.620	0.500	0.044	0.044	0.005
	Female	0.055	0.013	40.873	0.875	0.056	0.046	0.008
Fw1	Male	0.025	0.010	47.650	0.994	0.025	0.017	0.007
	Female	0.013	0.006	47.228	0.984	0.014	0.012	0.002
Fw2	Male	0.011	0.015	43.930	0.915	0.012	0.011	0.002
	Female	0.014	0.014	46.031	0.957	0.014	0.012	0.002

Note: For trait definitions refer to Figs. 1A, 1B. ME is measurement error, ME5 is repeatability $((MS_{S\times I} - MS_{err})/(MS_{S\times I} + MS_{err}))$, and col. is column.

*Computed as $0.798\sqrt{(MS_{S\times I} - MS_{err})}$, where MS is mean square, because the number of replicate measurements is two.

†An estimate of fluctuating asymmetry (FA) including ME ($0.798\sqrt{MS_{S\times I}}$).

‡Mean |R – L| of untransformed measurements, where R is right side and L is left side.

Table 3. Results (unscaled fluctuating asymmetry) from ANOVA analyses of |log(R/L)| variation, where R is right side and L is left side, of all traits and both sexes in the Chilean bee *Xeromelissa rozeni*.

Source of variation	df	Mean square	*P*
(*a*) All traits and both sexes included.			
Trait	6	0.301	**1.00×10^{-11}**
Sex	1	0.004	0.462
Trait × sex	8	0.006	0.380
Error	833	0.006	
(*b*) Wings, both sexes.			
Sex	1	0.003	0.837
Trait	1	5.32×10^{-5}	0.19
Sex × trait	1	0.0003	0.336
Error	161	0.0003	
(*c*) MxpTotal, both sexes.			
Sex	1	2.37×10^{-10}	0.997
Error	89	2.32×10^{-5}	
(*d*) MxpTotal and Fw2, both sexes.			
Trait	1	0.004	**0.036**
Sex	1	1.24×10^{-5}	0.502
Sex × trait	1	1.26×10^{-5}	0.73
Error	171	1.06×10^{-4}	
(*e*) Mxp5.5 only, both sexes.			
Sex	1	0.03	**0.003**
Error	96	0.003	
(*f*) Fw2, both sexes.			
Sex	1	7.0×10^{-6}	0.722
Error	74	0.001	

Note: For trait definitions refer to Figs. 1A, 1B. Values in boldface type are significant ($P < 0.05$).

was low as demonstrated by high repeatabilities of FA (50%–99.9%) (Table 2*b*, column 15) except for Mxp5.5 in females (41%).

Differences in FA among groups

Observed FA variation was comparable between combined (two-way ANOVA) and separate (one-way ANOVA) analyses on traits and sexes (Tables 3*a*–3*f* presents the results of the two-way ANOVA and those one-way ANOVAs that gave significant *P* values). FA varied significantly among traits ($P = 1.0 \times 10^{-11}$; Table 3*a*) but not sexes ($P = 0.462$; Table 3*a*) and the pattern of variation was rather complex (Fig. 2, Tables 3*a*–3*f*). All of the significant main effects (trait and sex) and the significant interactions are by-products of four principal differences (significant values quoted below remain so after Bonferroni correction). First, apical palpomeres Mxp5 and Mxp6 along with the membranous portion between them (Mxp5.5) exhibited the highest levels of FA (FA10b ranging from 0.023 to 0.076; Table 2*b*, column 12). Second, the membranous portions of the palpomeres exhibited higher FA than the sclerotized parts (FA10b ranging from 0.032 to 0.076 in membranous compared with 0.005 to 0.034 in sclerotized traits). Third, significantly higher levels of FA were observed in Fw2 when analyzed separately with MxpTotal ($P = 0.036$; Table 3*d*). Fourth, a notable FA difference was observed between sexes in Mxp5.5 ($P = 0.003$; Table 3*e*; Fig. 2).

Discussion

Although somewhat controversial, the idea that increased heterozygosity is associated with reduced FA has found some support (Smith 1982; Leary et al. 1983, 1985*a*; Clarke et al. 1986; Vøllestad et al. 1999; Hosken et al. 2000). We have assessed FA in a series of mouthpart and wing traits in a bee species for which nectar acquisition is of greater than average importance than it is even in most other bees. *Xeromelissa rozeni* has enormously elongate maxillary palpi, especially palpomeres 3 and 4, that form an elongate tube for obtaining nectar from the deep nectaries of their floral hosts. With samples of both sexes, we expected greater FA in three instances (i.e., hypotheses): (1) males than females, (2) membranous compared with the sclerotized parts that link adjacent sclerotized parts of the palpomeres, and (3) in the palpomeres and wing variables other than MxP3 and MxP4 because of the importance of the latter in tube formation for obtaining nectar.

Contrary to our first hypothesis, there was no significant difference in the magnitude of FA between haploid males and diploid females for any of our variables except Mxp5.5, which did have higher FA in males than females (Tables 3*a*–3*f*). Although, overall this result was inconsistent with the hypothesis that females are under stronger selection for symmetry in nectar-acquiring structures than males due to their brood-provisioning activities, it is consistent with the idea of stronger selection against asymmetry in extreme traits (Soulé, and Couzin-Roudy 1982) and traits essential for survival (Crespi and Vanderkist 1997).

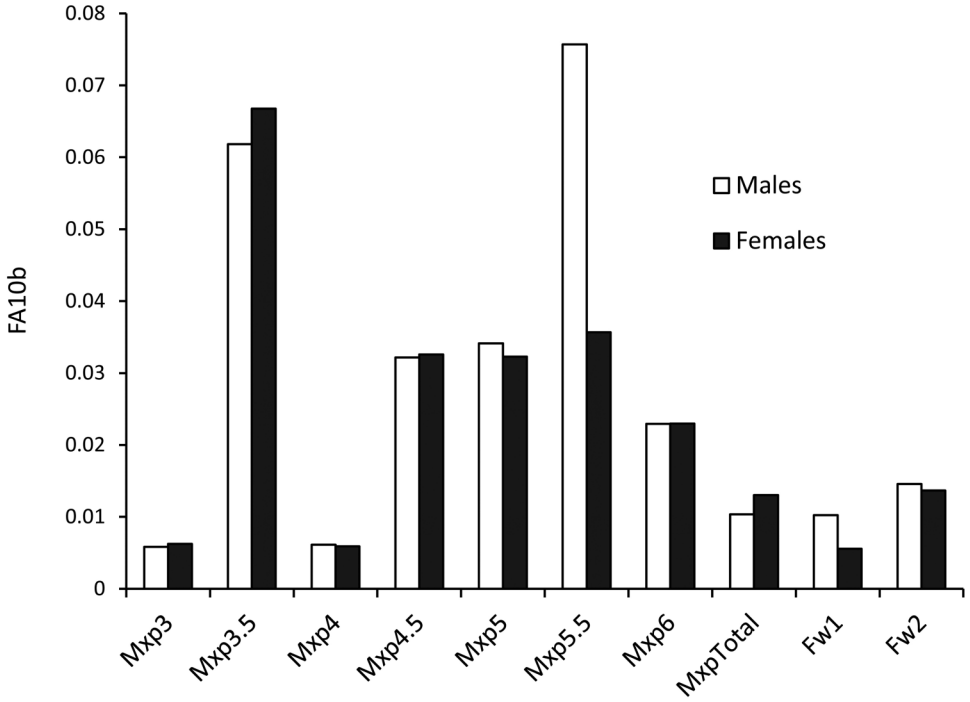
As expected (hypothesis 2), we found that FA was significantly higher in membranous parts Mxp3.5, Mxp4.5, and Mxp5.5 compared with sclerotized Mxp3, Mxp4, Mxp5, and Mxp6. There are three possible explanations for this. First, perhaps the membranous parts are "vestigial", due to their lack of function in feeding-tube formation (Michener 1995; Krenn et al. 2005). If this is the case, the results are consistent with the findings of Crespi and Vanderkist (1997), who observed higher levels of FA in vestigial wings of soldier thrips compared with fully functional wings of dispersers. Second, there could be reduced selection for symmetry in membranous regions because they might be used in a more plastic manner than the sclerotized regions. Third, the membranous regions might function to maintain broader scale symmetry in total maxillary palp lengths, lengthening on one side more than the other, to ensure closer approximation of the sclerotized parts throughout their lengths.

Our third hypothesis that MxP3 and MxP4 would have less FA than the other variables was only partially supported: they had less FA than other parts of the palpomeres, but had an equivalent amount to that found in the wing measurements. These results seem more due to the sclerotized palpomeres Mxp5 and Mxp6 having high levels of FA than low levels for MxP3 and MxP4. To explain this observation, we suggest that the apical two palpomeres might be nonfunctional in nectar uptake because they lack the specialized characteristics of Mxp3 and Mxp4, being cylindrical rather than transversely concave on their medial surfaces. Rather, we suspect that these parts may function more to help the apices of MxP4 to attain the nectar. This might be by assisting in gaining entry into the deeper portion of the nectaries, upward displacement of the nectar, or both.

Interestingly, MxP5 and successive palpomeres are "deciduous" in the congeneric species *Xeromelissa wilmattae* Cockerell, 1826 (Michener 1995); that is, these palpomeres are minute, tenuously attached and fall off, varying in number in different individuals and between sides within individuals (Michener 1995). Detailed observations of the mechanism of nectar uptake by these species in vivo would be useful, though likely difficult to carry out in the rigorous conditions of the Atacama Desert.

Upon inspection of the wing data, it was observed that Fw2 had significantly higher FA than Fw1 and MxpTotal. This once again shows that there is strong selection against asymmetry acting upon the maxillary palpi. Interestingly, the results would have been reversed if only one of the apical or membranous segments of the maxillary palpi were compared with the forewing traits. This suggests that wing venation symmetry may be important for this aerodynamically unusual bee that lives in a very dry and very windy environment. Our results, thus, support the view that trait selection is very important in FA studies, as various combinations of traits can yield quiet different results. We suggest that a larger number of traits

Fig. 2. Unscaled FA10b index values for all maxillary palpi and forewing traits of both sexes of the Chilean bee *Xeromelissa rozeni* representing asymmetry values with measurement error factored out.



be tested in detailed FA studies rather than the mean of 3.88 (SD = 1.53) traits tested in insect FA studies cited herein.

Gene duplication can be a way for haplodiploid organisms to avoid the issues associated with haploidy. The honeybee genome contains more duplicated gene sequences than other diploid insects, such as Diptera (The Honey Bee Genome Sequencing Consortium 2006; Simola et al. 2013). In addition to the honeybee, Simola et al. (2013) also found a higher number of gene duplicates in ants, which are also haplodiploid. If this is also true for *X. rozeni*, it may help explain the lack of FA difference between males and females.

In conclusion, there seems to be no association between heterozygosity levels and the magnitude of FA in the maxillary palpi of *X. rozeni*. However, our results support the view that selection can be an important force in maintaining low levels of FA in essential traits.

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References

Allendorf, F.W., and Leary, R.F. 1986. Heterozygosity and fitness in natural populations of animals. *Edited by* M.E. Soulé. *In* Conservation biology: the science of scarcity and diversity. Sinauer Associates, Inc., Sunderland, Mass. pp. 57–76.

Borgia, G. 1980. Evolution of haplodiploidy: models for inbred and outbred systems. Theor. Popul. Biol. **17**(2): 103–128. doi:10.1016/0040-5809(80)90001-5. PMID:7404436.

Bruckner, D. 1976. The influence of genetic variability on wing symmetry in honeybees (*Apis mellifera*). Evolution, **30**(1): 100–108. doi:10.2307/2407677.

Cane, J.H. 1983. Chemical evolution and chemosystematics of the Dufour's gland secretions of the lactone producing bees (Hymenoptera: Colletidae, Halictidae and Oxaeidae). Evolution, **37**(4): 657–674. doi:10.2307/2407908.

Carter, A.J.R., Osborne, E., and Houle, D. 2009. Heritability of directional asymmetry in *Drosophila melanogaster*. Int. J. Evol. Biol. **2009**: 1–7. doi:10.4061/2009/759159.

Clarke, G.M. 1997. The genetic basis of developmental stability. III. Haplodiploidy: are males more unstable than females? Evolution, **51**(6): 2012–2028. doi:10.2307/2411023.

Clarke, G.M., Brand, G.W., and Whitten, M.J. 1986. Fluctuating asymmetry: a technique for measuring developmental stress caused by inbreeding. Aust. J. Biol. Sci. **39**(02): 145–153. doi:10.1071/BI9860145.

Clarke, G.M., Oldroyd, B.P., and Hunt, P. 1992. The genetic basis of developmental stability in *Apis mellifera*: heterozygosity versus genic balance. Evolution, **46**(3): 753–762. doi:10.2307/2409643.

Cranford, T.W. 1992. Directional asymmetry in the odontocete forehead. Am. Zool. **32**(5): 140A.

Crespi, B.J., and Vanderkist, B.A. 1997. Fluctuating asymmetry in vestigial and functional traits of a haplodiploid insect. Heredity, **79**: 624–630. doi:10.1038/hdy.1997.208.

Cruickshank, R.H., and Thomas, R.H. 1999. Evolution of haplodiploidy in Dermanyssine mites (Acari: Mesostigmata). Evolution, **53**(6): 1796–1803. doi:10.2307/2640441.

Fernández, M.J., Springthorpe, D., and Hedrick, T.L. 2012. Neuromuscular and biomechanical compensation for wing asymmetry in insect hovering flight. J. Exp. Biol. **215**(20): 3631–3638. doi:10.1242/jeb.073627. PMID:22771747.

Graham, J.H., Freeman, D.C., and Emlen, J.H. 1993. Antisymmetry, directional asymmetry, and dynamic morphogenesis. Genetica, **89**: 121–137. doi:10.1007/978-94-011-0830-0_10.

Graham, J.H., Raz, S., Hel-Or, H., and Nevo, E. 2010. Fluctuating asymmetry: methods, theory, and applications. Symmetry, **2**(2): 466–540. doi:10.3390/sym2020466.

Hall, B.K. 2005. Fifty years later: I. Michael Lerner's *Genetic Homeostasis* (1954)—a valiant attempt to integrate genes, organisms and environment. J. Exp. Zool. Part B Mol. Dev. Evol. **304B**(3): 187–197. doi:10.1002/jez.b.21052.

Hosken, D.J., Blanckenhorn, W.U., and Ward, P.I. 2000. Developmental stability in yellow dung flies (*Scathophaga stercoraria*): fluctuating asymmetry, heterozygosity and environmental stress. J. Evol. Biol. **13**(6): 919–926. doi:10.1046/j.1420-9101.2000.00239.x.

Huang, Y.C., Nipitwattanaphon, M., Lee, C.C., Keller, L., and Wang, J. 2014. Mapping a novel sex determination gene in ants. *In* 17th Congress of the International Union for the Study of Social Insects (IUSSI), Cairns, Australia, 13–18 July 2014. IUSSI. pp. 13–18.

Krenn, H.W., Plant, J.D., and Szucsich, N.U. 2005. Mouthparts of flower-visiting insects. Arthropod Struct. Dev. **34**(1): 1–40. doi:10.1016/j.asd.2004.10.002.

Kudo, S., and Mori, S. 2000. Ploidy does not affect fluctuating asymmetry in the carpenter bee (Hymenoptera: Anthophoridae). Can. Entomol. **132**(3): 333–335. doi:10.4039/Ent132333-3.

Leary, R.F., Allendorf, F.W., and Knudsen, K.L. 1983. Developmental stability and enzyme heterozygosity in rainbow trout. Nature, **301**: 71–72. doi:10.1038/301071a0. PMID:6823283.

Leary, R.F., Allendorf, F.W., and Knudsen, K.L. 1984. Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. Am. Nat. **124**(4): 540–551. doi:10.1086/284293.

Leary, R.F., Allendorf, F.W., and Knudsen, K.L. 1985*a*. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution, **39**(6): 1318–1326. doi:10.2307/2408788.

Leary, R.F., Allendorf, F.W., Knudsen, K.L., and Thorgaard, G.H. 1985*b*. Heterozygosity and developmental stability in gynogenetic diploid and triploid rainbow trout. Heredity, **54**: 219–225. doi:10.1038/hdy.1985.29. PMID:4008275.

Lerner, I.M. 1954. Genetic homeostasis. Oliver and Boyd, Edinburgh. [American edition, 1954, John Wiley & Sons, New York. Reprinted 1970, Dover Publications, New York.]

Michener, C.D. 1995. A classification of the bees of the subfamily Xeromelissinae (Hymenoptera: Colletidae). J. Kans. Entomol. Soc. **68**(3): 332–345.

Owen, R.E. 2012. Applications of morphometrics to the Hymenoptera, particularly bumble bees (*Bombus*, Apidae). *In* Morphometrics. *Edited by* C. Wahl. InTech, Rijeka, Croatia. pp. 1–30.

Owen, R.E., and Packer, L. 1994. Estimation of the proportion of diploid males in populations of Hymenoptera. Heredity, **72**: 219–227. doi:10.1038/hdy.1994.31.

Palmer, A.R. 1994. Fluctuating asymmetry analyses: a primer. *In* Developmental instability: its origins and evolutionary implications. *Edited by* T.A. Markow. Springer Science+Business Media B.V., Dordrecht, the Netherlands. pp. 335–364. doi:10.1007/978-94-011-0830-0_26.

Palmer, A.R. 1996. Waltzing with asymmetry. BioScience, **46**(7): 518–532. doi:10.2307/1312930.

Palmer, A.R., and Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annu. Rev. Ecol. Syst. **17**: 391–421. doi:10.1146/annurev.es.17.110186.002135.

Palmer, A.R., and Strobeck, C. 2003. Fluctuating asymmetry analyses revisited. *In* Developmental instability: causes and consequences. *Edited by* M. Polak. Oxford University Press, New York. pp. 279–319.

Pratt, A.E., and McLain, D.K. 2002. Antisymmetry in male fiddler crabs and the decision to feed or breed. Funct. Ecol. **16**(1): 89–98. doi:10.1046/j.0269-8463.2001.00605.x.

Rohlf, F.J., and Sokal, R.R. 1995. Statistical tables. W.H. Freeman, San Francisco, Calif.

Rozen, J.G., Jr. 2003. A new tribe, genus, and species of South American panurgine bee (Andrenidae: Panurginae), oligolectic on *Nolana* (Nolanaceae). Appendix: Evidence for the phylogenetic position of *Nolanomelissa* from nuclear EF-1α sequence data by John S. Ascher. *In* Apoidea Neotropica: Homenagem aos 90 Anos de Jesus Santiago Moure. *Edited by* G.A.R. Melo and I. Alves-dos-Santos. Editora UNESC, Circiúma, Brazil. pp. 93–108.

Rozen, J.G., Jr., and Wyman, E.S. 2015. The Chilean bees *Xeromelissa nortina* and *X. sielfeldi*, their nesting biologies and immature stages, including biological notes on *X. rozeni* (Colletidae: Xeromelissinae). Am. Mus. Novit. No. 3838.

Simola, D.F., Wissler, L., Donahue, G., Waterhouse, R.M., Helmkampf, M., Roux, J., Nygaard, S., Glastad, K.M., Hagen, D.E., Viljakainen, L., Reese, J.T., Hunt, B.G., Graur, D., Elhaik, E., Kriventseva, E.V., Wen, J., Parker, B.J., Cash, E., Privman, E., Childers, C.P., Muñoz-Torres, M.C., Boomsma, J.J., Bornberg-Bauer, E., Currie, C.R., Elsik, C.G., Suen, G., Goodisman, M.A.D., Keller, L., Liebig, J., Rawls, A., Reinberg, D, Smith, C.D., Smith, C.R., Tsutsui, N., Wurm, Y., Zdobnov, E.M., Berger, S.L., and Gadau, J. 2013. Social insect genomes exhibit dramatic evolution in gene composition and regulation while preserving regulatory features linked to sociality. Genome Res. **23**(8): 1235–1247. doi:10.1101/gr.155408.113. PMID:23636946.

Smith, D.R., Crespi, B.J., and Bookstein, F.L. 1997. Fluctuating asymmetry in the honeybee, *Apis mellifera*: effects of ploidy and hybridization. J. Evol. Biol. **10**(4): 551–574. doi:10.1046/j.1420-9101.1997.10040551.x.

Smith, M.H. 1982. Genetic variability and antler growth in a natural population of white-tailed deer. *In* Antler development in Cervidae. *Edited by* R.D. Brown. Caesar Kleberg Wildlife Research Institute, Kingsville, Tex. pp. 365–388.

Soulé, M.E., and Couzin-Roudy, J. 1982. Allometric variation. 2. Developmental instability of extreme phenotypes. Am. Nat. **120**(6): 765–786.

The Honeybee Genome Sequencing Consortium. 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature, **443**: 931–949. doi:10.1038/nature05260. PMID:17073008.

van Wilgenburg, E., Driessen, G., and Beukeboom, L. 2006. Single locus complementary sex determination in Hymenoptera: an "unintelligent" design? Front. Zool. **3**(1): 1–15. doi:10.1186/1742-9994-3-1. PMID:16393347.

Vøllestad, L.A., Hindar, K., and Møller, A.P. 1999. A meta-analysis of fluctuating asymmetry in relation to heterozygosity. Heredity, **83**: 206–218. doi:10.1046/j.1365-2540.1999.00555.x. PMID:10469209.

Woolf, C.M., and Markow, T.A. 2003. Genetic models for developmental homeostasis: historical perspectives. *In* Developmental instability: causes and consequences. *Edited by* M. Polak. Oxford University Press, New York. pp. 99–115.

Zayed, A. 2004. Effective population size in Hymenoptera with complementary sex determination. Heredity, **93**: 627–630. doi:10.1038/sj.hdy.6800588. PMID:15354193.

Zayed, A., and Packer, L. 2005. Complementary sex determination substantially increases extinction proneness of haplodiploid populations. Proc. Natl. Acad. Sci. U.S.A. **102**(30): 10742–10746. doi:10.1073/pnas.0502271102. PMID:16020532.