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Trade-offs in the evolution of bumblebee colony and body size: a comparative analysis

Raúl Cueva del Castillo[1], Salomón Sanabria-Urbán[1] & Martín Alejandro Serrano-Meneses[2]

[1]Lab. de Ecología; UBIPRO, Universidad Nacional Autónoma de México, FES Iztacala. A. P. 314, 54090 Edo. México, México
[2]Laboratorio de Biología Evolutiva, Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Carretera Tlaxcala-Puebla Km. 1.5, 90062 Tlaxcala, México

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Correspondence
Raúl Cueva del Castillo, Lab. de Ecología, UBIPRO, Universidad Nacional Autónoma de México, FES Iztacala. A. P. 314, 54090 Edo. México, México.
Tel: +52 55 5623-1228
Fax: +52 55 5623-1223
E-mail: rcueva@ecologia.unam.mx

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Abstract

Trade-offs between life-history traits – such as fecundity and survival – have been demonstrated in several studies. In eusocial insects, the number of organisms and their body sizes can affect the fitness of the colony. Large-than-average body sizes as well as more individuals can improve a colony's thermoregulation, foraging efficiency, and fecundity. However, in bumblebees, large colonies and large body sizes depend largely on high temperatures and a large amount of food resources. Bumblebee taxa can be found in temperate and tropical regions of the world and differ markedly in their colony sizes and body sizes. Variation in colony size and body size may be explained by the costs and benefits associated with the evolutionary history of each species in a particular environment. In this study, we explored the effect of temperature and precipitation (the latter was used as an indirect indicator of food availability) on the colony and body size of twenty-one bumblebee taxa. A comparative analysis controlling for phylogenetic effects as well as for the body size of queens, workers, and males in bumblebee taxa from temperate and tropical regions indicated that both temperature and precipitation affect colony and body size. We found a negative association between colony size and the rainiest trimester, and a positive association between the colony size and the warmest month of the year. In addition, male bumblebees tend to evolve larger body sizes in places where the rain occurs mostly in the summer and the overall temperature is warmer. Moreover, we found a negative relationship between colony size and body sizes of queens, workers, and males, suggesting potential trade-offs in the evolution of bumblebee colony and body size.

Introduction

Trade-offs represent the costs paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another (Stearns 1989). The resources diverted to one trait may reduce the resources canalized to other traits, thus constraining adaptive evolution (see Roff and Fairbairn 2007; Kingsolver and Diamond 2011). In several studies, trade-offs between life-history traits such as fecundity and survival have been demonstrated (Gustafsson et al. 1994; Sinervo and DeNardo 1996; Berger et al. 2008; Saglam et al. 2008; Cox et al. 2010; Gilbert and Manica 2010). In social organisms, in addition to the individual traits associated with fitness, there are costs and benefits associated with group-living, which can shape the evolution of such taxa (Cartar and Dill 1990; Baer and Schmid-Hempel 2003; Strohm and Bordon-Hauser 2003; Lopez-Vaamonde et al. 2009; Dornhaus et al. 2012). Living in social groups entails two opposing selective pressures for their members: (1) competition over limited resources and (2) the benefits that living in groups provide (Williams 1992).

In some senses, in eusocial insects, a colony can be considered analogous to an individual. The number of organisms (Strohm and Bordon-Hauser 2003; Tibbetts and Reeve 2003; Dornhaus et al. 2012) and their body sizes (Cnaani and Hefetz 1994; Jeanne and Nordheim 1996; Kapustjanskij et al. 2007) can affect colony productivity. Very commonly, the individuals in a colony perform different functional roles. These roles are associated with role-specific selective regimes that favor genetic integration of traits specific to each role and the adaptive divergence of role-specific phenotypes (Kovacs et al. 2010). In the social hymenopterans, females are divided into queen and worker castes (Wilson 1971). Queens are females specializing on reproduction, and the workers rear and protect the offspring and carry provisions to the nest. In eusocial bees, the sexes are similar in morphology but differ in size, with females generally being larger than males (Stubblefield and Seger 1994; Gadagkar 1996). Males serve only to inseminate females and are both smaller and shorter lived than workers or queens (Stubblefield and Seger 1994). Body size polymorphisms in contemporary social insect species are evidence of past selection favoring different optimal body sizes within castes and sexes (Stubblefield and Seger 1994). For example, the larger size of queens probably reflects selection for higher fecundity and for higher mating success (Kovacs et al. 2008). Similarly, male size may reflect selection for increased mating success (Amin et al. 2012).

Bumblebees are often described as a primitive eusocial group, because their social organization is simpler than that of other honeybees (Gadagkar 1996), and workers retain their reproductive potential (Michener 1974). In *Bombus*, with the exception of few tropical taxa, each colony contains only one queen (i.e., monogyny; Michener 1974). There are two important differences between bumblebees and honeybees: first, the size of the colonies of bumblebees is substantially smaller (70–1800 individuals) than the honeybees (>20,000 individuals); and second, with a few exceptions, the former are annual organisms. Fertilized queens emerge from their hibernacula in late winter or early spring and establish new nests for a first generation of workers that will help grow the colonies. Once the queen has established a colony of workers, her main activity is to lay more eggs, while the workers maintain the colony and forage for food (for details, see Alford 1975). Depending on the species considered, after producing the first generation of workers, at some point in the spring or summer, the queen biases her offspring production in favor of new queens and males, which leave the colony after maturation. The nest rapidly degenerates once males and young queens leave. In geographic areas, where the spring is very short, the queen rears only one batch of workers before commencing the production of reproductive individuals (Goulson 2010).

In bumblebees (*Bombus* sp.), adult body size depends on the amount of food received (Sutclifffe and Plowright 1988, 1990), although developing queens and males require more food over a longer period compared to worker larvae. As in other eusocial bees, queens tend to be larger than both males and workers and store large quantities of fat that are consumed during their hibernation (Richards 1946; Cumber 1949; Pereboom 2001). Large size can improve the hibernation possibilities of queens, increase their fecundity, and may grant them an advantage in the competition for nesting places (Owen 1988; Müller and Schmid-Hempel 1992). Aside from the differences in size and fat storage, the workers are very similar to queens in external morphology. Large individuals can improve the nest's thermoregulation (Bishop and Armbruster 1999) and foraging efficiency (Cnaani and Hefetz 1994; Kapustjanskij et al. 2007). At least in some species, larger males have an advantage in male–male competition (Alcock and Alcock 1983; Williams 1991; Paxton 2005).

A large colony and a large body size of colony members depend largely on the high availability of food resources (Sutclifffe and Plowright 1988, 1990; Pereboom et al. 2003; Pelletier and McNeil 2003), whereas larvae and pupae incubated under warmer temperatures can grow larger than those developed under lower temperatures (Goulson 2010). Bumblebees from cold climates are larger than bumblebees from temperate ones; however, the latter are smaller than tropical species (Peat et al. 2005). Arctic species tend to be larger than southern species, perhaps due to the thermoregulatory advantages of large body size (Pekkarinen 1979). Nonetheless, body size could be related to a higher extent to the size of food rewards to be harvested, than to the energetic advantages of thermoregulation (Heinrich 1993).

There is considerable variation in colony size among bumblebee taxa. In some, a nest can produce less than one hundred individuals, whereas in others, more than one thousand bees are produced. Arctic species have a short reproductive season and may produce only a brood of workers before producing the sexual brood. On the other hand, in the tropics, the annual cycle is absent, and bumblebees can have large colonies (Sakagami 1976; Husband 1977). A large colony can be adaptive. In general, colony size correlates positively with bumblebees' foraging range (Goulson 2010); therefore, a large colony may obtain more food resources than a smaller one (Pelletier and McNeil 2003). A large food supply means a larger workforce that will provide more help, including food provisioning, brood care, and defense of the colony, as well as a subsequent increased production of sexuals (Owen et al. 1980; Müller and Schmid-Hempel 1992). Although a large colony can be adaptive, its size can be constrained by the availability of resources. Large colonies may deplete the resources that are closer to them (Goulson 2010), which may result in a limited amount of resources available to invest in offspring production (Roulston and Cane 2002; Pelletier and McNeil 2003; Quezada-Euán et al. 2011). Also, the size of the colony can be constrained by parasitism (Schmid-Hempel et al. 1990; Otti and Schmid-Hempel 2008), and the ability of the queen to maintain control of the workers (Strohm and Bordon-Hauser 2003; Amsalem and Hefet 2011; Zanette et al. 2012).

In this study, we have explored the relationship between colony size and the body size of queens, workers, and males in twenty-one bumblebee species. Young queens and males represent the reproductive success of the colony, and these are also the most energetically demanding individuals, whereas workers maintain the colony. Because a colony with access to a large amounts of food resources may keep growing (Pelletier and McNeil 2003), and because the larvae of bumblebees that receive large amounts of food and are incubated under warmer temperatures can attain large body sizes (Sutclifffe and Plowright 1988, 1990; Pereboom et al. 2003; Couvillon and Dornhaus 2009; Goulson 2010), bumblebee taxa with large colonies and large body size are expected to occur in environments with high temperatures and large availability of food resources (see Sakagami 1976; Laverty and Plowright 1985; Peat et al. 2005). Nonetheless, the variation in their colony size (i.e., number of members) as well as the body size of the members of a colony may be explained by the costs and benefits associated with the evolutionary history of each species in a particular environment. The resources diverted to one trait may reduce the resources canalized to other traits, thus constraining adaptive evolution (see Roff and Fairbairn 2007). If a colony has limited resources to use for offspring production, this can generate a trade-off between the number and the body size of the members of the colony.

Methods

We performed a bibliographic search for information on the nests' bionomy, considering colony size and body size of *Bombus* taxa (Table 1). We collected information on colony size from the literature by executing a search on Google Scholar using the terms "colony size," "colony collection," "bionomy" or "worker number," "bee or bumblebee" to find colony sizes. Google Scholar was used as the search engine because it catalogs the full-text versions of published papers. We considered colonies collected in natural environments, but because information on wild bumblebee colonies is extremely limited (see

Table 1. Average values for colony size, thorax, and head width of males, queen, and workers for 21 Colonial *Bombus* species.

Species	Colony size (# workers)	References	Males		Queens		Workers	
			Thorax width	Head width	Thorax width	Head width	Thorax width	Head width
Bombus ardens	43.0	15	4.65	3.91**,***	7.08	4.98**,***	4.98	3.72**,***
Bombus atratus	54.3	3	3.53	3.24*	5.66	3.75*	3.92	2.89*
Bombus brasilensis	42	8	3.70	3.30 (2)	8.50	5.83****	6.45	4.95****
Bombus diversus	271.5	15	4.85	3.85**	7.14	5.05**	4.79	3.95**
Bombus ephippiatus	465.0	10	3.38	2.76*	5.08	3.40*	3.64	2.74*
Bombus honshuensis	40.0	13	4.50	3.70**	6.10	4.80**	4.40	3.50**
Bombus huntii	515	11	3.13	2.43*	4.66	3.30*	3.22	2.44*
Bombus hypocrita	38.0	6, 15	5.78	4.49**	7.74	5.63**	5.36	4.18**
Bombus ignitus	62.5	15	6.40	4.90**	8.10	5.80**	6.30	4.80**
Bombus impatiens	465	4	3.42	2.59*	5.21	3.69*	3.38	2.67*
Bombus lucorum	121.2	12	4.16	2.96*	4.40	3.13*	3.75	2.83*
Bombus medius	800	7	3.24	2.59*	5.66	3.94*	3.64	2.82*
Bombus melaleucus	43	18	8.5	5.2 (1)	10.39	6.71 (4)	6.80	4.45 (6)
Bombus morio	67.5	9	3.65	2.60*	5.93	4.16 (6)	4.32	3.04 (7)
Bombus pennsylvanicus	150	16	4.28	3.80*	5.44	3.82*	3.96	3.06*
Bombus pseudobaicalensis	20	15	4.80	3.80***	6.50	4.70***	4.60	3.70***
Bombus pullatus	336.5	5, 19	4.03	3.15*	5.90	4.05*	4.50	3.16*
Bombus schrencki	25.5	15	4.50	2.60***	7.90	5.50***	4.80	4.00***
Bombus terrestris	150.0	2	7.76	4.39*,***	7.86	5.47*,***	4.77	3.97*,****
Bombus transversalis	118.6	14, 17	5.38	3.80 (2)	9.23	6.06 (9)	5.18	3.55 (7)
Bombus vosnesenskii	1848	1	2.98	2.56*	5.30	3.67*	3.41	2.51*

All the units are expressed in mm. Sample sizes from data not included in publications are shown between parentheses. * = Cueva del Castillo and Fairbairn 2012; ** = Inoue and Yokoyama (2006); *** = Inoue et al. (2008); **** = Laroca (1972). Colony size reported with no decimal spaces was estimated using one colony

1: Allen et al. (1978); 2: Buttermore (1997); 3: Gonzalez et al. (2004); 4: Husband (1977); 5: Janzen (1971); 6: Katayama and Takamizawa (2004); 7: Michener and La Berge (1954); 8: Laroca (1972); 9: Laroca (1976); 10: Laverty and Plowright (1985); 11: Medler (1959); 12: Müller and Schmid-Hempel (1992); 13: Ochiai and Katayama (1982); 14: Olesen (1989); 15: Sakagami and Katayama (1977); 16: Shelly et al. (1991); 17: Dornhaus and Cameron (2003); 18: Hoffmann et al. (2004); 19 Hines et al. (2007).

Waters et al. 2010), for *Bombus lucorum,* we also considered five reproductive colonies reared from queens caught from natural environments and transferred to the field after the emergence of approximately five to ten first workers (see Müller and Schmid-Hempel 1992), which were below the workers number limits to consider a field colony in this study. We considered the mean number of workers per colony as an indicator of relative colony size, which is likely to be determined by both genetic and ecological parameters (Plowright and Pallett 1979; Pomeroy and Plowright 1982; Gerloff and Schmid-Hempel 2005; Herrmann et al. 2007), and it is correlated with the number of queens and males in a colony (Pomeroy and Plowright 1982). Considering bumblebees' reproductive cycle, in order to reduce the risk of underestimating colony productivity due to initial growing phases, dispersal, and/or mortality, we omitted those nest reports considered by the authors as early phases of development, senescent colonies, or colonies with 10 workers or less (see Sakagami and Katayama 1977). As in other social insects, colony size across bumblebee taxa is very difficult to find (rev. in Dornhaus et al. 2012); thus, we found sufficient information on colony size for only 22 bumblebee taxa. However, the information of one species (*Bombus deuteronymus*) was not considered because it was not possible to get information on body size. Of the 21 taxa considered in the study, 12 of these (57%) had available information for two or more nests (see Table 2). The average sample size in the data set is only three colonies per species, which is similar to the average found for other social species (see Dornhaus et al. 2012).

We used head width (maximal distance between the distal surfaces of the eyes, measured dorsally) and thorax width (intertegular distance) as indicators of body size. Most of the body size measurements can be found in published papers (see Laroca 1972; Inoue and Yokoyama 2006; Inoue et al. 2008; Cueva del Castillo and Fairbain 2012), but we also included the unpublished measurements of four other species: *Bombus brasilensis, Bombus tranversalis, Bombus melaleucus* and *Bombus morio*. Individuals of these taxa were measured from collections at the following museums: Museum of Entomology of the University of California at Riverside (UCR) and Berkeley, the Natural History Museum of Los Angeles (NHM-LA), the California Academy of Science and The Natural History Museum of Paris. In these cases, measurements were taken considering the methods reported by Cueva del Castillo and Fairbairn (2012).

Given that temperatures and food availability can affect bumblebees' colony and adult body size, and because bumblebee taxa can be found in an extensive geographic distribution, we considered the average regional temperatures and precipitation parameters associated with the available information on collecting places of museum specimens considered by Fairbairn and Cueva del Castillo (2012), unpublished information from these museums, and published sources (Laroca 1972; Inoue and Yokoyama 2006; Inoue et al. 2008), and the reported nest

Table 2. Collecting places for the nests of 21 bumblebee taxa. Sample sizes are shown between parentheses.

Species	Collecting place	Collecting month
Bombus ardens	Honshu Island, Japan (5)	May, June
Bombus atratus	Facatativa, Colombia (5); Loja, Ecuador (2)	February, April, July
Bombus brasilensis	Antonina, Parana, Brazil (1)	February
Bombus diversus	Honshu Island, Japan (8)	June, July, August, September
Bombus ephippiatus	Monteverde and Volcan Irazu, Costa Rica (2)	February, July
Bombus honshuensis	Honshu Island, Japan (6)	July, August, October
Bombus huntii	Ruidoso New Mexico, USA (1)	August
Bombus hypocrite	Honshu Island, Japan (5)	July, August
Bombus ignitus	Honshu Island, Japan (8)	June, July, August, September
Bombus impatiens	Michigan, USA (1)	August
Bombus lucorum	Basel, Switzerland (5)	April
Bombus medius	San Luis Potosi, Mexico (1)	June
Bombus melaleucus	El Porvenir; Colombia (1)	Unreported
Bombus morio	Alexandra, Parana, Brazil (2)	February
Bombus pennsylvanicus	Willcox, Arizona, USA (1)	August, September
Bombus pseudobaicalensis	Hokkaido Island, Japan (1)	August
Bombus pullatus	San Vito de Java, Estación Biológica Pitilla, Costa Rica (2)	June, July
Bombus schrencki	Hokkaido Island, Japan (2)	August, September
Bombus terrestris	Hobart, New Zealand (2)	February, October
Bombus transversalis	Napo River, Afiangu, Ecuador (1); Tambopata River, Peru (2)	April, October
Bombus vosnesenskii	Tilden Park, California, USA (1)	June

collecting places (Table 2). As moisture affects flowering (Rathcke and Lacey 1985), precipitation was used as an indicator of food availability (see similar rationale in Yom-Tov and Geffen 2006; Branson 2008). We searched for the geographic coordinates for each bumblebee specimen and nest using the software Google Earth (http://earth.google.com/). Then, using BioClim, we calculated climate variables (Busby 1991). BioClim estimated nineteen climatic parameters; however, we only considered climatic variables associated with high temperatures and precipitation. Once we calculated the climatic information, the average of these parameters was estimated for each species (see Table 3).

Phylogeny

We used *Bombus* DNA sequences (available from GenBank) of five genes to determine the phylogenetic relationships among the 21 taxa included in the comparative analysis: mitochondrial 16S rRNA (16S) and protein-encoding nuclear genes arginine kinase (ArgK), elongation factor-1 alpha F2 copy (EF-1α), long-wavelength rhodopsin copy 1 (Opsin), and PEPCK (phosphoenolpyruvate carboxykinase). Additionally, we included sequences of *Bombus auricomus*, *Bombus soroeensis*, and *Bombus waltoni* as outgroups, because these taxa are early diverging lineages in the *Bombus* phylogeny (Cameron et al. 2007). GenBank accession numbers are given in Table S1.

The sequences of every gene were aligned separately using the program CLUSTAL X (ver. 2.0.12; Larkin et al. 2007). After alignment, the sequences were edited so that every species had the same number of positions. Uncertain alignment and gap regions were removed from each the data set. The individual gene fragments had the following number of aligned sites: 16S, 458; ArgK, 754; EF-1α, 660; Opsin, 619; and PEPCK, 679. The combined (five genes) data set included 3170 bp.

Nuclear gene sets were partitioned into exon and intron regions, as in Cameron et al. (2007). The model of evolution of each gene partition was selected based on Akaike's information criteria as implemented in jModelTest (ver. 2.1.1; Darriba et al. 2012). The model parameters used for each gene partition were as follows: GTR+I+G for 16S and EF-1α exon; GTR+I for ArgK exon and Opsin exon; GTR+G for EF-1α intron; GTR for Opsin intron; HKY for ArgK intron; HKY+G for PEPCK exon and PEPCK intron.

Bayesian inference of phylogenetic relationships among species was implemented in MrBayes (see. 3.1.2; Ronquist and Huelsenbeck 2003). Six independent analyses (runs) were carried out for the combined data set (8,000,000 generations, sampling trees every 1000 generations, four chains with mixed models and flat priors). All trees estimated prior to stationarity were discarded (burnin). A consensus tree (Fig. 1) was estimated from the six independent analyses in MrBayes. Before mixing the runs, we

Table 3. Average climatic parameters estimated using BioClim software for the nest collecting places of 21 colonial *Bombus* species.

	Temperature (°C)				Precipitation (mm)			
Species	Annual	Warmest month	Warmest trimester	Wettest trimester	Annual	Rainiest month	Rainiest trimester	Warmest trimester
Bombus ardens	15.7	30.4	24.9	22.4	1615.0	215.0	536.0	497.0
Bombus atratus	20.7	29.0	22.8	22.0	1553.0	300.3	790.0	317.6
Bombus brasilensis	23.0	30.9	25.4	24.1	1122.8	182.8	493.4	356.4
Bombus diversus	9.2	25.7	19.8	19.6	1836.8	256.4	714.7	700.8
Bombus ephippiatus	18.7	29.3	20.81	19.9	1040.1	220.4	586.6	364.4
Bombus honshuensis	5.6	23.8	17.8	14.4	1349.0	151.0	420.0	403.0
Bombus huntii	10.4	30.0	20.0	7.3	402.8	59.7	166.3	77.4
Bombus hypocrite	13.3	30.4	24.2	24.1	1372.7	196.7	517.3	504.3
Bombus ignites	12.7	29.3	23.2	23.2	1448.0	199.0	592.0	592.0
Bombus impatiens	13.6	30.1	23.4	19.1	1220.1	131.3	372.5	360.9
Bombus lucorum	4.7	20.8	15.1	10.5	530.3	72.9	201.9	138.3
Bombus medius	22.8	31.7	25.1	24.4	1930.9	365.5	970.8	576.6
Bombus melaleucus	15.5	22.5	16.9	16.4	1561.7	247.4	642.1	405.6
Bombus morio	12.6	29.0	22.0	19.8	1005.9	153.5	419.2	206.1
Bombus pennsylvanicus	16.5	32.2	25.6	21.3	1182.4	148.4	402.7	383.5
Bombus pseudobaicalensis	−0.5	15.6	10.8	8.4	1444.0	196.0	517.0	510.0
Bombus pullatus	22.8	29.5	23.8	22.8	3202.3	473.1	1217.0	617.7
Bombus schrencki	5.9	21.5	16.6	14.7	189.0	165.0	409.0	392.0
Bombus terrestris	6.9	22.0	16.8	14.1	330.7	151.0	274.2	351.1
Bombus transversalis	24.3	30.3	24.9	24.3	2417.5	312.6	863.1	570.6
Bombus vosnesenskii	10.2	25.9	17.6	4.2	965.5	167.1	471.4	55.9

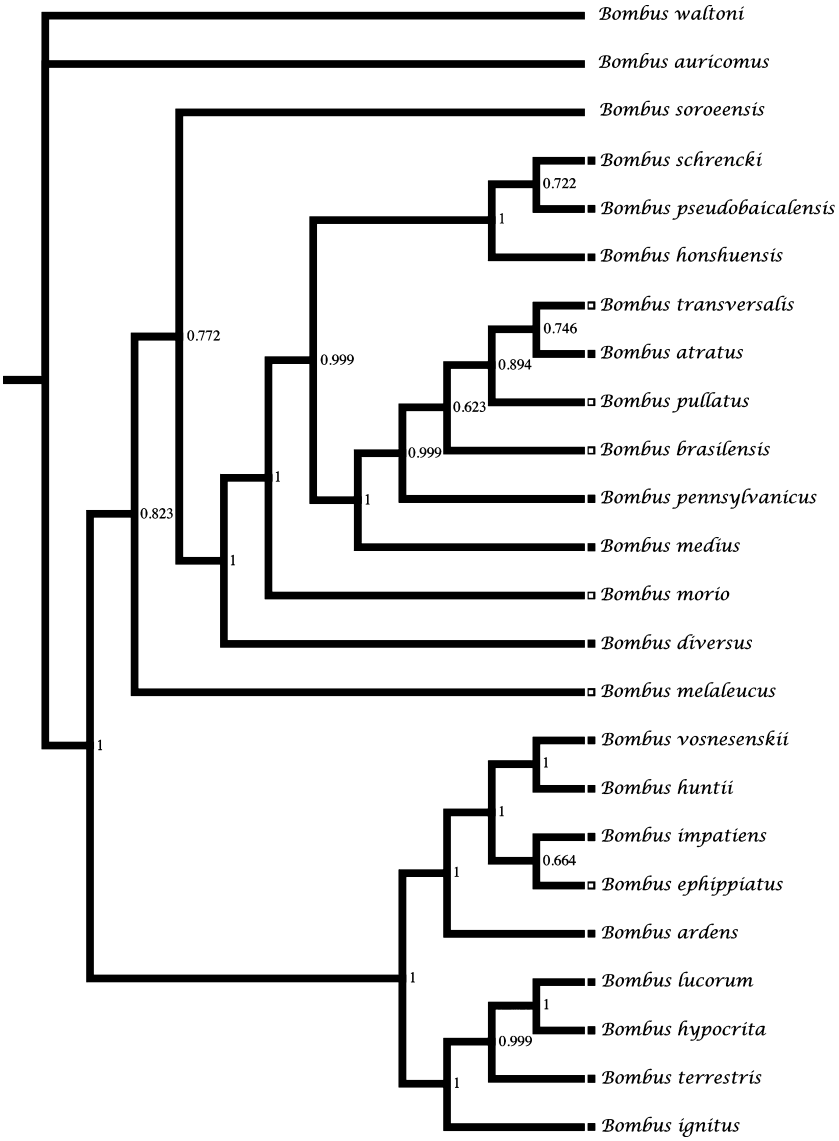

Figure 1. Consensus tree topology of 21 bumblebee taxa (see Table 1) used in the study (see Methods). Bayesian posterior probability values are shown for each node. Black squares denote taxa from temperate habitats, whereas white squares denote taxa from tropical habitats. Branch lengths are available from Data S1.

checked the convergence of their log-likelihood values in Tracer 1.2 (Rambaut and Drummond 2003). With the exception of the positioning of *B. morio* and *Bombus pullatus*, the resulting phylogenetic tree was consistent with Cameron et al.'s (2007) tree. In our phylogenetic tree, *B. morio* appears more basal, compared to Cameron et al.'s (2007) tree. Similarly, *B. pullatus* appears relatively more basal, closer to *Bombus pennsylvanicus* than to *Bombus transversalis* (Fig. 1).

Comparative analyses

To test how (1) the body size of queens, workers, and males correlated with colony size and (2) to test whether the body size of queens, workers, and males correlated with climatic variables, we used the PGLS method (phylogenetic generalized least squares; Pagel 1997, 1999; Garland and Ives 2000; Freckleton et al. 2002). PGLS is a phylogenetic comparative method that incorporates the phylogenetic autocorrelation of the data in the structure of errors (variance–covariance matrix, Martins and Hansen 1997; Freckleton et al. 2002). The structure of variance–covariance matrix was determined from the phylogeny (Fig. 1). PGLS was used to test the maximum likelihood of the evolutionary regression coefficient between traits (Pagel 1997, 1999). To improve the fit of our data to the models, we estimated the maximum likelihood of the weighting parameter λ (lambda; see Freckleton et al. 2002) and used it to correct for phylogenetic effects in all linear models (Pagel 1997, 1999). All PGLS models were produced using the package "caper" (Orme et al. 2012) as implemented in R (ver. 3.0.1; R Development Core Team 2013). Prior to analyses, the three species used as outgroups were pruned from the consensus tree using the "na.omit" function implemented in "caper." This procedure allowed us to preserve the original branch lengths of the consensus tree.

The body sizes of queens, workers, and males were summarized using the R function "phyl.pca," which performs phylogenetic PC (principal components) analysis (Revell 2009). PC 1 was used as an indicator of general body size, which explained approximately 97% of variance (Table 4). Previous to comparative analyses, morphological parameters were log10-transformed.

To evaluate the impact of climatic variables on bumblebee colony size and body size, we performed four multiple PGLS models. We used a backward elimination modeling approach starting with a global model; we used a stepwise approach to eliminate nonsignificant terms until a minimum adequate model with significant terms was reached. In the first model, we regressed colony size (independent variable) on the climatic parameters (8 variables; see Table 3), and later produced a minimum adequate model. Second, in order to test the potential trade-off between colony size and body size of queens, workers, and males, and given that the climatic parameters may indirectly affect body size by influencing colony size, we built three global models considering the effect of all climatic parameters on the body sizes of each caste/sex class. We later eliminated nonsignificant terms until a minimum adequate model with significant terms was reached. In a final step, we incorporated colony size (as a predictive variable) to the reduced models; with this procedure, we sought to reduce the predictive power of the climatic variables on body size, hence testing which term best explains the body size of each caste. Thus, we regressed the body size of queens, workers, and males (independent variables, respectively) on the climatic parameters and colony size. We used one-tailed probabilities for hypothesis testing because each one of our *a priori* hypotheses is directional.

Table 4. Phylogenetic principal components analyses (PPCA) of two morphometric characters of queens, workers, and males of 21 *Bombus* taxa. Eigenvectors of the two PC (principal components) are shown.

	Queens		Workers		Males	
λ	0.001		0.667		0.430	
Variable	PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
Thorax width	0.707	−0.707	0.707	−0.707	0.707	−0.707
Head width	0.707	0.707	0.707	0.707	0.707	0.707
Eigen value	1.981	0.018	1.970	0.029	1.878	0.121

Results

Climatic parameters and colony size

The multiple phylogenetic regressions indicated that the climatic parameters exhibit a significant relationship with colony size. After eliminating the nonsignificant terms, only the warmest month and the rainiest trimester of the year remained in the model ($r^2 = 0.428$, $F_{2,18} = 8.467$, $P < 0.001$). Colony size showed a positive association with the warmest month ($\beta = 4.920$, SE = 1.373, $t = 3.583$, $P < 0.001$) and a negative association with the rainiest trimester of the year ($\beta = -1.821$, SE = 0.513, $t = -3.550$, $P < 0.001$). Interestingly, a λ value of zero indicates that ecological factors, rather than phylogenetic, affect these relationships and the relationships between colony size and body size. The result suggests that colonies tend to reach larger sizes when there are increases in overall temperature, as well as decreases in overall precipitation of the rainy season occur.

Climatic parameters and colony size-body size trade-off

Queens

After elimination of nonsignificant terms (climatic variables; see Methods), the warmest month and warmest trimester of the year remained in the model. This multiple phylogenetic regression on climatic parameters was highly significant (r^2: 0.294, $F_{3,18} = 5.159$, $P = 0.005$, $\lambda = 0.000$). However, once colony size was added to the model (see Methods), the climatic parameters were no longer significant (warmest month: $\beta = -3.300$, SE = 2.843, $t = -1.161$, $P = 0.131$; warmest trimester: $\beta = 3.233$, SE = 2.264, $t = 1.428$, $P = 0.086$). In addition, and according to our hypothesis, the relationship between colony size and body size between body size and the number of individuals in bumblebee colonies resulted negative and marginally significant ($\beta = -0.217$, SE = 0.108, $t = -2.001$, $P = 0.031$).

Workers

After elimination of nonsignificant terms, the warmest month and warmest trimester of the year remained in the model. This multiple phylogenetic regression on climatic parameters was highly significant (r^2: 0.305, $F_{3,18} = 5.386$, $P = 0.004$, $\lambda = 0.000$). The effect of the climatic parameters on workers' body size exhibited a similar pattern to the one exhibited by queens. Nonetheless, once colony size was added to the model, the climatic parameters were no longer significant (warmest month: $\beta = -1.782$, SE = 1.997, $t = -0.892$, $P = 0.192$; warmest trimester: $\beta = 1.865$, SE = 1.590, $t = 1.173$, $P = 0.129$). Nevertheless, a negative and significant relationship between colony size and body size was found ($\beta = -0.222$, SE = 0.077, $t = -2.912$, $P = 0.005$, Fig. 2).

Males

After elimination of nonsignificant terms, the precipitation on warmest trimester the year remained in the model. This multiple phylogenetic regression on climatic parameters was highly significant: r^2: 0.245, $F_{2,19} = 7.488$, $P = 0.002$, $\lambda = 0.823$. In this case, precipitation during the warmest trimester of the year showed a positive and significant effect on male body size ($\beta = 0.318$, SE = 0.132, $t = 2.412$, $P = 0.013$). Similar to queens and workers, males showed a negative and significant relationship between colony size and body size ($\beta = -0.178$, SE = 0.078, $t = -2.289$, $P = 0.017$, Fig. 2).

Discussion

After controlling for phylogenetic effects, our results indicated that in bumblebees climatic parameters affect the size of the colonies, as well as the body size of the males. We found a negative association between colony size and the rainiest trimester, and a positive association between the colony size and the warmest month of the year. In addition, male bumblebees tend to evolve larger body sizes in places where the rain occurs mostly in the summer and the overall temperature is warmer. Also, we found a negative association between colony size and the body size of queens, workers, and males, which suggests a macro evolutionary trade-off between colony size and bumblebee body size (Fig. 2).

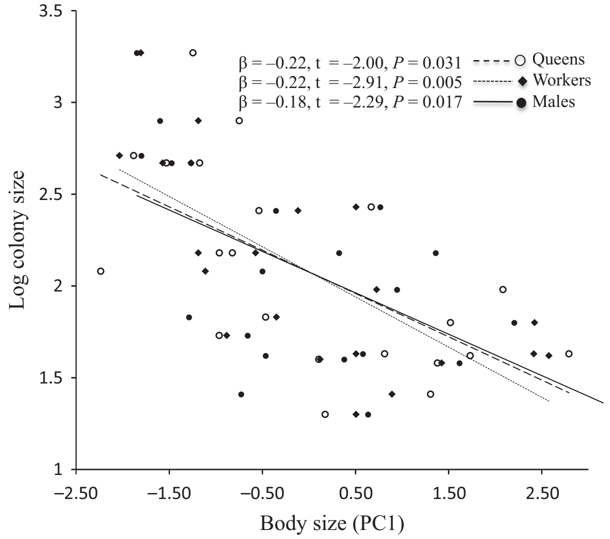

Figure 2. Relationships between body size (principal component 1) of queens, workers, and males of 21 bumblebee species and their colony size (see Table 1). Ordinary least squares regressions fitted are shown for illustrative purposes. Inserted statistics refer to the corresponding phylogenetically corrected models.

There is little information on the relationship between climatic parameter and colony size in social insects (Purcell 2011). Nonetheless, our results contrast with those obtained in attine ants, in which temperatures and precipitation did not have a significant effect on the colony size of these species (Ferguson-Gow et al. 2014).

Arctic bumblebee species have a short reproductive season and may produce only a brood of workers before producing the sexual brood. In contrast, in the tropics, the annual cycle is absent, and bumblebees can have large colonies (Sakagami 1976; Husband 1977). Perhaps the colonies of tropical species may keep growing because they have access to a large amount of food resources (Pelletier and McNeil 2003). Nonetheless, despite the strong effect that precipitation has on food availability (Yom-Tov and Geffen 2006; Branson 2008), we found that taxa with large colonies are associated with places with low overall precipitation during the rainy season, as well as a warm summer.

The phenology of bumblebees is strongly related to flower phenology, and the peak of abundance of bumblebees is strongly related to the abundance of flowers (Pyke et al. 2011). Temperature and moisture affect the availability of flowers (Rathcke and Lacey 1985). In temperate regions, frost in spring and autumn may limit the flowering season. On the other hand, in tropical forests, many species flower more than once a year (Croat 1975; Opler et al. 1976; Putz 1979), and food is available all year around. In neotropics, many herbs and shrubs flower in the rainy season (Croat 1975; Monasterio and Sarmiento 1976), and tree species flower in the dry and the rainy season (Frankie et al. 1974). However, heavy rains can constrain the foraging activity of bumblebees, as well as the volume and concentration of nectar, which may also be influenced by temperature and wind (Pyke et al. 2011). Thus, foraging constraints and nectar dilution must lead to a compensatory increase in food consumption (Wrigth 1988) via an increase in foraging activity and energy expenditure (Wrigth 1988; Lotz and Nicolson 1999) that could limit the relative colony profits and its size in places with heavy rains. In addition, under low temperatures, there is a reduction in colony size due to lower rates of eclosion, associated with an increase in workers' thermoregulation and broad incubation times (Vogt 1986b; Gardner et al. 2007). The percentage of bees maintaining the brood increase as population increases (Vogt 1986a), and perhaps, large colonies generate enough heat as a by-product of nonthermoregulatory activity (Seeley and Heinrich 1981). The plasticity of bumblebee body size could be more related to food availability than to the energetic costs of thermoregulation (Heinrich 1993). Interestingly, male body size but not female size (queens and workers) was positively affected by the warmest and wettest period of the year. In wet, tropical climates precipitation is likely to influence nectar availability; therefore, larger (and thus more energetically costly) bees may be expected to occur (Peat et al. 2005). Perhaps due to individual requirements or total biomass, the body size of males may indicate food availability for the colony during the growing season (see Heinrich 1993). The males are produced in larger numbers than the queens, and queens require more food during their development than males. Nonetheless, the gynes (young queens) receive more food during their development than the larvae of males and workers (Goulson 2010). On the other hand, the workers could be energetically less demanding than the reproductive members of the colony. However, they are also produced in larger numbers than queens (see Lopez-Vaamonde et al. 2009). In any case, bumblebee adult body size is proportional to the amount of food received (Sutclifffe and Plowright 1988, 1990), and a reduction in food availability could eventually have an impact on the size that colony members can reach, generating a trade-off. This trade-off may depend on which resources limit parental investment (Rosenheim et al. 1996), or a combination of genetic and environmental effects (Fjerdingstadt 2005).

Because individuals in a colony are not genetically identical, there are RA (relatedness asymmetries; see Shik 2008), which cause conflict over reproductive allocation (Hamilton 1964) that can translate into the number and/or size of the colony members. In small societies like those of many *Bombus* taxa, worker reproduction and the capacity of a worker to replace the queen are strongly selected. Workers will have few contenders, and a high chance of replacing the queen. Moreover, there is intense conflict over reproduction (Alexander et al. 1991; Bourke 1999). The subordinates, which are morphologically similar to the queens, should constantly threaten the queen reproductively, and the queen must be active maintaining her dominant position. However, if there is an increase in colony size, the reproductive potential of the workers drops, due to a reduction in their chances of replacing the queen, and an increase in the coincidence of interests of workers and queens (Alexander et al. 1991; Bourke 1999). As large colonies evolve, the division of labor becomes more complex, as workers and queens act to maximize inclusive fitness (Ferguson-Gow et al. 2014), and the degree of dimorphism between queens and workers arises due to high specialization on the role of workers (Bourke 1999; Ferguson-Gow et al. 2014).

This comparative analysis provides limited insights into how RA shapes sex allocation conflict. However, two lines of evidence may suggest that the trade-off in the evolution of bumblebee colony and body size could be mainly the result of environmental constrains rather than a sex allocation conflict on RA: (1) queens, workers, and males showed a similar negative relationship between colony size and body size (Fig. 2), which suggests that limitation of resources could affect the body size of queens, workers, and males in a similar way and (2) a phylogenetic comparative study on the allometric relationships of queens on workers body size of 70 species of bumblebees showed an isometric relationship between both castes. No deviations from isometry constitute evidence that reproductive selection (Cueva del Castillo and Fairbairn 2012), or specialization on workers, has not accelerated divergence on females, as suggested by RA conflict. Nonetheless, there is no information for colony size for many of the 70 bumblebee species studied by Cueva del Castillo and Fairbairn (2012). However, from these, twenty-one show large variation in colony size.

Interestingly, ecological factors, rather than phylogenetic, are affecting the relationships between the size of queens, workers, males, and colonies with the climatic parameters, suggesting that these traits can be plastic (Field et al. 2010). Between-species variation in colony and body size may be explained by the costs and benefits associated with the history of each species in a particular environment. We must point out that we could be underestimating the trade-off differences among the colony members due to differences in the number and biomass of queens, workers, and males. In any case, because colony and body size data from each species came from different sources, and despite the small amount of data on the colonies (i.e., sample size; Table 2), the negative relationship between bumblebees' colony and body size may suggest that in nature, this trade-off could be very strong and may constrain the evolution of colony and body size in bumblebees, as it may also do in ants (Shik 2008) and other eusocial insects.

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Conflict of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Phylogenetic tree used in the study. Branch lengths are included.

Table S1. GenBank accession numbers for the *Bombus* species and out groups considered to build the phylogeny used for comparative analysis (see Cameron et al. 2007).