



Climatic variation across a latitudinal gradient affect phenology and group size, but not social complexity in small carpenter bees

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Abstract

Greater social complexity at lower latitudes has been observed in a variety of arthropods from termites to spiders. Social behavior in the small carpenter bees, *Ceratina*, has been shown to vary widely both between species and across geographic range. Our goal was to determine how social plasticity of three populations of *Ceratina* species, *C. calcarata* and *C. strenua*, vary across a latitudinal gradient. The longer rearing season in the south did not result in two separate brood rearing periods, but instead increased brood production of a single brood with a higher female sex bias. The social structure of nests remained stable across both species' ranges: mothers exhibit prolonged parental care and worker dwarf eldest daughters occur among populations and species. This is the first report of worker daughters in *C. strenua*. The ubiquity of worker daughter production in eastern North American *Ceratina* suggests that factors outside of climate underlie the early division of labor between the reproductive mother and worker dwarf eldest daughter.

Keywords Social evolution · Maternal investment · Developmental plasticity · Geographic distribution · *Ceratina*

Introduction

Physiological characteristics can vary drastically across latitudinal gradients as organisms adapt to climatic variations (Roff, 1992; Stearns 1992). Thermal clines may lead to conspicuous phenotypic plasticity and/or local adaptation across the geographic range of a wide range of ectothermic species (Huey and Kingsolver 1989; Knies et al. 2009; Schulte et al. 2011). Bergmann's clines, for instance, denote variations in body size within species which are positively correlated with latitude and altitude (Bergmann, 1847). Among insects, the period during which reproduction and growth can occur increases at lower latitudes and altitudes

(Bradshaw and Holzapfel 2007; Gullan and Cranston 2010). Such extension of the active season may, in turn, allow for increased development time and greater brood production. For example, the species range of the large carpenter bee (*Xylocopa virginica*) is limited by active season length: at higher latitudes, the active season is too short for complete brood development (Skandalis et al. 2011). By contrast, an extended active season at lower latitudes may lead to the adoption of a bivoltine life cycle (Roff 1980; Davison and Field 2017), as observed in crickets (Masaki 1972; Mousseau and Roff 1989), butterflies (Nygren et al. 2008), and moths (Välimäki et al. 2013). As such, in addition to phenology, a species' behavior and physiology can also vary considerably across latitudinal clines. These data comparing physiology and phenology across latitudinal gradients can then be used to predict the effect of climate change on population range shifts (Parmesan and Yohe 2003).

Studies of the effects of latitudinal gradients on social species have revealed the importance of ecological pressures in the evolution of social behavior (Purcell 2011; Field et al. 2010). Highly social behavior or eusocial behavior requires the overlap of more than one generation in the nest (Wilson 1975; Oster and Wilson 1978; Michener 1985; Hunt and Amdam 2005), which occurs more frequently in the warmer, more stable environments of the tropics (Purcell

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2011). On a global scale, Gaston et al. (1996) found a higher proportion of eusocial Hymenoptera in Costa Rica than the UK. Similarly, at regional scales among termites, colony size increases in populations that occur closer to the equator (Porter and Hawkins, 2001). Similar trends were observed in social spiders, where social species of the genus *Anelosimus* occur only in the tropics, while subsocial and nonsocial species occur at higher latitudes (Aviles 1997; Aviles et al. 2007; Agnarsson 2006).

Social polymorphisms across a latitudinal gradient are common in the halictine bees, in which many species demonstrate increased social complexity at low latitudes and altitudes (Plateaux-Quénu 2008; Field et al. 2010). Populations of *Augochlorella aurata* are solitary in the northern part of their range, but frequently form eusocial groups in more temperate areas (Packer 1990; Mueller 1996). By comparison, social complexity in *Halictus rubicundus* and *LasioGLOSSUM calceatum* varies by altitude, with solitary populations found at higher altitudes and eusocial populations at lower altitudes (Sakagami and Munakata 1972; Eickwort et al. 1996). Interestingly, a reverse trend of increased sociality at higher latitudes has been observed in some species. Across five subfamilies of ants (Kaspari and Vargo 1995) and swarm-founding wasps (Jeanne 1991), colony size has been shown to increase at higher latitudes. The number of foundresses and opportunities for sib rearing increases at higher altitudes in many species of allodapine bees, including *Exoneura robusta* and *E. angophorae* (Schwarz et al. 1997; Cronin and Schwarz 1999a). On a smaller scale, research on multiple species has revealed the influence of microhabitat differences, on social polymorphism (Hirata and Higashi 2008). Only by examining the social structure of additional species with differing life cycles and ecological pressures can we gain a full understanding of the effects of climatic variation on the development of highly social behaviors.

The small carpenter bees, *Ceratina*, provide a phylogenetic contrast and independent origin of sociality within the bee family Apidae; there is one origin of eusociality in the subfamily Apinae (corbiculate bees) and a second independent origin of eusociality in their sister subfamily Xylocopinae (carpenter bees). Ecological factors selecting for social traits vary across arthropods, insects and Hymenoptera, but it remains unknown if similar ecological pressures led to the repeated origins of sociality in the bees. *Ceratina* offer unique insights into the origin and elaboration of social behavior because of their full range of social behavior from solitary to eusocial within the genus (Michener 1985; Rehan and Toth 2015). The previous work has described in detail many life history traits of the eastern North American small carpenter bee, *C. calcarata* (Rehan and Richards 2010a, 2010b, Rehan et al. 2014). At the northern extent of its range, the group exhibits two of the three hallmarks of

eusocial behavior: overlapping generations and reproductive division of labor (Rehan and Richards 2010a; Rehan et al. 2014). In late spring, females build nests within dead broken stems. The mother hollows the stem to lay eggs and then provides each offspring with its own nutrition-rich pollen ball. The first egg laid is typically female and receives less pollen and nutrition from the mother than subsequent offspring (Rehan and Richards 2010b; Lawson et al. 2016). In addition to this nutritional manipulation by the mother, when the offspring emerges the mother will use physical force to coerce the first daughter to leave the nest to forage and help collect provisions for her nestmates (Rehan and Richards 2013). This dwarf eldest daughter does not have the opportunity to reproduce and serves only as a worker for the colony, leading to a reproductive division of labor (Rehan et al. 2014). Because of the short summer and limited brood rearing season experienced by populations in Ontario and New Hampshire, there is no opportunity for a second brood in which to provide cooperative brood care, the third hallmark of eusocial behavior. In the lower latitudes of its range, however, *C. calcarata* populations have a prolonged active season and larger clutches (Rau 1928; Grothaus 1962; Chandler 1975; Kislow 1976; Johnson 1988). If populations at the southernmost extent of *C. calcarata*'s range produce multiple generations per year, this could present opportunities for a second brood and cooperative brood care. To examine the role of climatic variation on physiology and behavior in the small carpenter bee, we compared colony demography, maternal investment, and social structure across a latitudinal gradient in two sympatric and closely related species of *Ceratina*: *C. calcarata* and *C. strenua*.

Materials and methods

Nest collections

Nests of *C. calcarata* and *C. strenua* were collected from dead broken stems of sumac (*Rhus* spp.) and raspberry (*Rubus* spp.) from three populations: near Durham, New Hampshire (NH), USA (43.1339°, - 70.9264°), Lake Ozark, Missouri (MO), USA (38.1986°, - 92.6388°) and Athens, Georgia (GA), USA (33.9519°, - 83.3576°). *Ceratina calcarata* nests were collected weekly in New Hampshire, but *C. strenua* was not found in this location. Nests of both species were collected monthly in Missouri and Georgia. All nests were collected before 0800 h or after 1800 h to ensure nests were occupied and adult bees were not out foraging. Collections took place from May until September. Specifically, the population in New Hampshire was sampled daily on May 4–7, 13–14, June 3–5, 8–12, 16–19, 22–30, July 2–3, 6–7, 16–17, 28–29, August 2–4, 10, 16, 24, and September 1, 7, 15, 19–20, 2015. Missouri was sampled twice

daily from May 2–9, June 8–16, July 11–18, August 8–15, and September 8–14, 2016. In Georgia, nests were collected daily May 8–30, June 1–13, 25–29, July 2–5, 13–15, 22–24, 29–30, August 5, 10–12, and September 12–15, 2016. Bees were transported in their nests from collection sites to the University of New Hampshire at 4 °C and stored at 4 °C until processing.

Nest processing

Nests were dissected lengthwise and nest contents recorded, including brood developmental stages, individual brood cell position, with the inner most brood cell position as brood cell one, total number of brood cells, nest width and length, and the presence of an adult female who was assumed to be the mother. Nest contents dictated assignment of a nest class for each colony (Rehan and Richards 2010a). Founding nests (FN) have newly excavated pith and contain a single adult female, the mother, but no brood. Active brood nests (AB) contain at least one pollen ball, indicating that the brood is not complete and the mother is still actively provisioning for and laying eggs. Full brood nests (FB) contain a larva or pupa in the outermost brood cell. Because an egg takes about 5 days to hatch (Johnson 1988), the presence of the larva indicates the mother has likely completed her clutch. Mature brood nests (MB) contain only adult bees, including a mother and her recently eclosed offspring (Rehan and Richards 2010a).

Measurements of adult bees included head width (which is an accurate measure of total body size) and wing wear (a proxy for age and foraging effort; Rehan and Richards 2010a). Adults were stored at –20 °C. Brood, including eggs, larvae, and pupae were stored in PCR tubes in an incubator at 25 °C with 50% humidity until eclosion. Offspring development was recorded every other day. Larval development was scored by comparing the length of the larva relative to the pollen mass and pupal development was scored by eye and body pigmentation (Rehan and Richards 2010a). Brood sex and head width were recorded for all offspring that reached adulthood. Sex was determined by counting the number of metasomal terga; females have six segments, while males have seven (Rehan and Richards 2010a). Lab-reared adults were stored at –20 °C. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Climate data

To match collection dates, climate data from May through September 2015 were obtained from the National Oceanic and Atmospheric Administration records for the weather station in Durham, NH (43.1432°, –70.9501°) and for May through September 2016 for Lakeside, MO (38.2043°,

–92.6232°) and Athens, GA (33.948°, –83.3275°) (<http://www.noaa.gov>). To compare overall climatic patterns, cumulative degrees above 25 °C and cumulative rainfall in centimeters were recorded.

Statistical analysis

All statistical analyses were performed in JMP v.7.01 (SAS, Cary, NC, USA). Temperature, precipitation, clutch size, brood development, maternal head width, and offspring head width were all separately tested with the Shapiro–Wilk’s test for normality and Levene’s test for equality of variance. Maximum temperature, rainfall, maternal head width, clutch size, offspring head width and days in each developmental stage were analyzed using a Kruskal–Wallis test followed by a post-hoc Wilcoxon each pair test (Sokal and Rohlf 1995). Sex ratio was calculated as percent male and compared using a Chi-squared goodness-of-fit test. A linear regression was used to compare maternal head width to clutch size, sex ratio, and offspring head width, and offspring head width to brood cell position. All reported *p* values are two-tailed.

Results

Climatic variation

Nests of *C. calcarata* and *C. strenua* were collected across their range in New Hampshire, Missouri, and Georgia (Fig. 1). A total of 203 *C. calcarata* nests and 0 *C. strenua* nests were collected from New Hampshire, 79 *C. calcarata* and 235 *C. strenua* nests from Missouri, as well as 137 *C. calcarata* and 481 *C. strenua* nests from Georgia. The collection locations occur in three distinct climate zones. The maximum daily temperature at the New Hampshire site is significantly cooler during the *Ceratina*’s active season (May–September), while the Georgia site is significantly warmer overall (Kruskal–Wallis test $\chi^2 = 124.41$, $df = 2$, $p < 0.0001$; Fig. 2a). Though the Missouri site had the most cumulative rainfall (measured as millimeters of rainfall daily), its rainfall was not significantly different from the sites in New Hampshire or Georgia (Kruskal–Wallis test $\chi^2 = 2.46$, $df = 2$, $p = 0.29$; Fig. 2b).

Population dynamics

The nesting phenology of *C. calcarata* and *C. strenua* varies across their range. While the start of *C. calcarata*’s nesting season is delayed by a month in New Hampshire relative to the timing of nesting in Missouri and Georgia, overall reproductive phenology follows similar patterns of progression through the colony cycle (Fig. 3). *Ceratina calcarata* mothers establish their nests before May in both Missouri

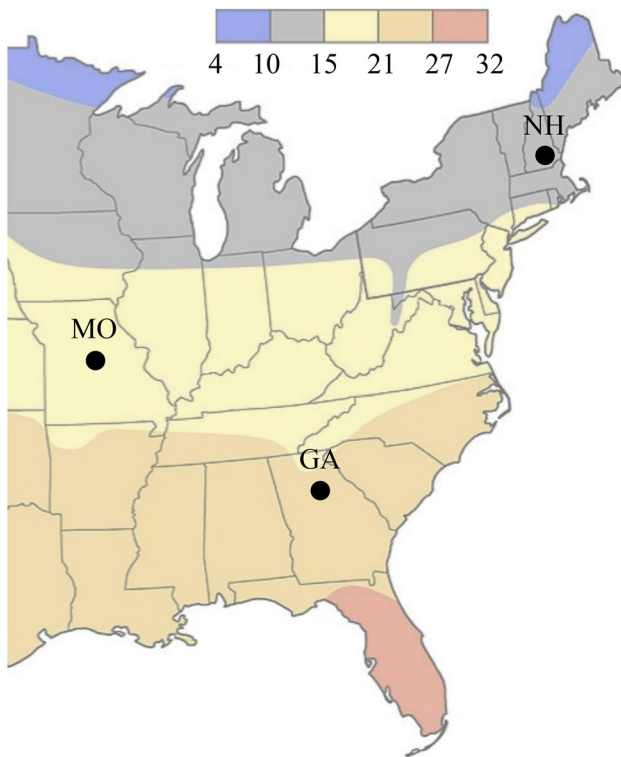


Fig. 1 Collection locations of *Ceratina* species in New Hampshire (NH), Missouri (MO), and Georgia (GA). Background colored divisions are defined by annual summer temperature (°C) based on a map by NOAA's National Climatic Data Center. (Color figure online)

and Georgia, while in New Hampshire mothers must wait for the warmer May temperatures to begin their nests. In all populations, active brood *C. calcarata* nests occur for about 2 months, peaking in June in New Hampshire and Missouri, and May in Georgia. The nests are completed with a full brood by August in New Hampshire and by July in Missouri as well as Georgia and mature brood begin to eclose within the next few weeks. Some females will establish new nests late in the season in Missouri and Georgia, but there was no evidence of bivoltinism (a second round of active brood nests) in any population. The regional nesting phenology of *C. strenua* was similar to that of sympatric *C. calcarata*. The nesting cycle of *C. strenua* in Missouri begins about 1 month later than in Georgia, while the full brood nests peak in July in Missouri, and June in Georgia (Fig. 4). We found no evidence of bivoltinism in either *C. strenua* population.

Maternal physiology

Mothers of the New Hampshire population of *C. calcarata* ($N=203$; mean \pm sem, 2.07 ± 0.01 mm) are significantly larger than those of Missouri ($N=45$; 1.64 ± 0.04 mm) or Georgia ($N=85$; 1.77 ± 0.02 mm; Kruskal–Wallis test $\chi^2 = 163.51$, $df=2$, $p < 0.0001$; Table 1). Mothers of nests

in Georgia are significantly larger than those collected in Missouri ($p=0.013$). By contrast, *C. strenua* mothers collected in Missouri ($N=135$; 1.56 ± 0.02 mm) were significantly larger than mothers collected in Georgia ($N=257$; 1.45 ± 0.01 mm; Kruskal–Wallis test $\chi^2 = 73.38$, $df=1$, $p < 0.0001$; Table 1).

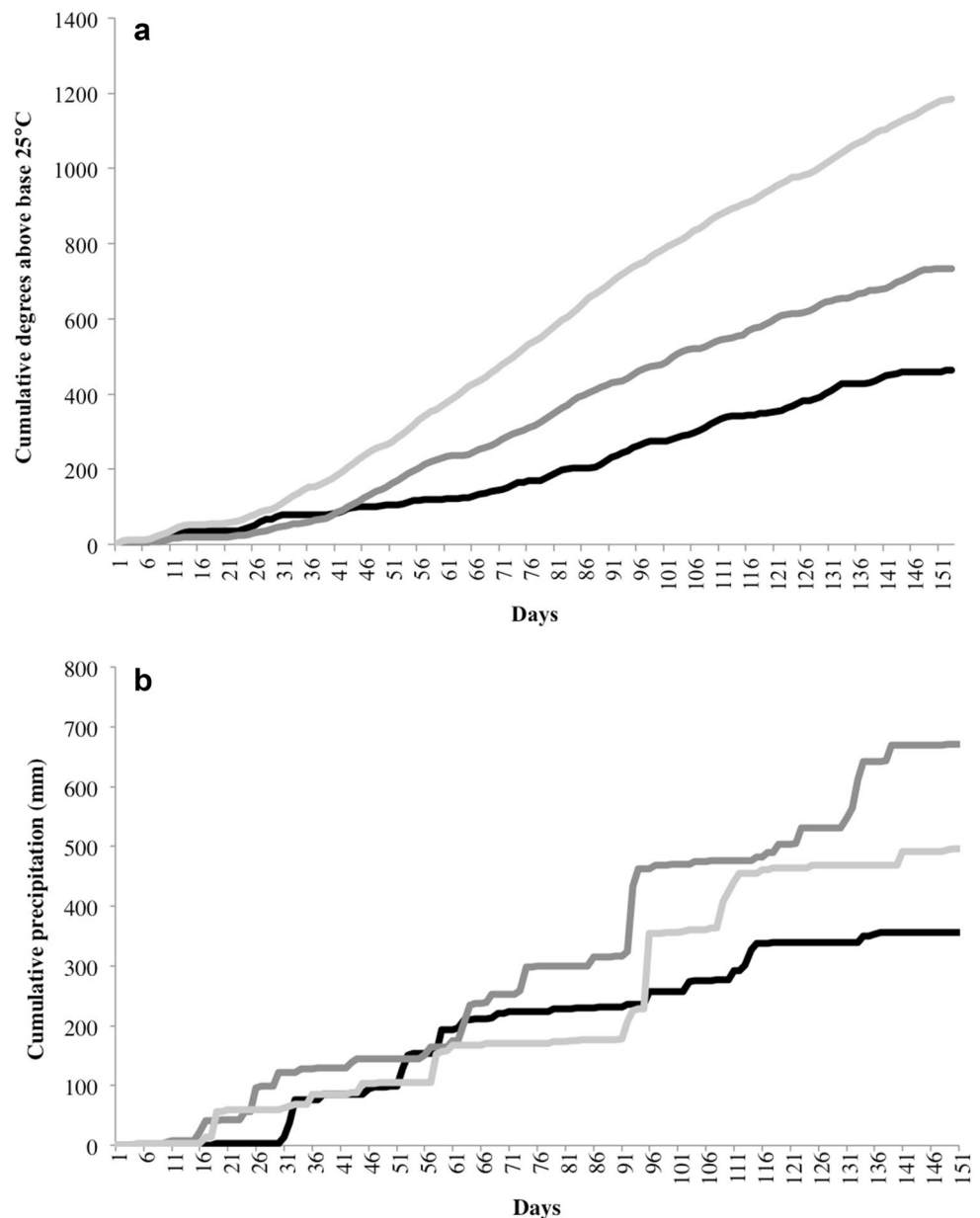
Clutch size

Average clutch size was calculated using only full brood nests to ensure nest completeness. The average clutch size of *C. calcarata* populations in New Hampshire ($N=142$; mean \pm sem, 8.71 ± 0.31) was significantly smaller than in populations in Missouri ($N=13$; 12.46 ± 0.94) or Georgia ($N=53$; 10.66 ± 0.66 ; Kruskal–Wallis test $\chi^2 = 14.94$, $df=2$, $p=0.0006$; Table 1), but the clutch size did not differ between Missouri and Georgia. The average clutch size of *C. strenua* was not significantly different between the Missouri ($N=24$; 10.79 ± 0.77) and Georgia populations ($N=147$; 10.64 ± 0.37 ; Kruskal–Wallis test $\chi^2 = 0.07$, $df=1$, $p=0.79$; Table 1). For *C. calcarata* in New Hampshire, larger mothers laid significantly larger clutches (Linear regression: $R^2=0.05$, $F=5.37$, $df=1$, $p=0.02$). Clutch size and maternal size were not correlated in Missouri ($R^2=0.19$, $F=1.63$, $df=1$, $p=0.24$) or Georgia ($R^2=0.03$, $F=1.44$, $df=1$, $p=0.23$), but it is noteworthy that relatively few *C. calcarata* nests were collected in these states. Maternal size was positively correlated with clutch size in *C. strenua* in both Missouri ($R^2=0.23$, $F=4.80$, $df=1$, $p=0.04$) and Georgia ($R^2=0.06$, $F=8.13$, $df=1$, $p=0.01$).

Nest sex ratio

Ceratina calcarata nest sex ratio was significantly more female-biased in Georgia ($N=55$ nests containing 307 offspring; male offspring = 43%) compared to nests in both Missouri ($N=27$ nests containing 113 offspring; 53% M) and New Hampshire ($N=94$ nests containing 253 offspring, 56% M; goodness-of-fit test $\chi^2 = 13.27$, $df=2$, $p=0.0013$; Table 1). The sex ratio of *C. strenua* had a similar trend to *C. calcarata*, where nests in Georgia ($N=258$ nests containing 660 offspring; 38% M) were significantly more female-biased compared to nests in Missouri ($N=72$ nests containing 217 offspring, 47% M; goodness-of-fit test $\chi^2 = 121.18$, $df=1$, $p < 0.0001$; Table 1). Sex ratio was not correlated with maternal head width for *C. calcarata* populations in New Hampshire or Missouri (NH: $R^2=0.003$, $F=0.20$, $df=1$, $p=0.66$; MO: $R^2=0.11$, $F=2.72$, $df=1$, $p=0.11$), but in Georgia larger mothers had significantly female-biased clutches ($R^2=0.08$, $F=4.08$, $df=1$, $p=0.05$). In *C. strenua* sex ratio was not correlated with maternal head width in any population examined (MO: $R^2=0.02$, $F=0.87$, $df=1$, $p=0.34$; GA: $R^2=0.01$, $F=2.14$, $df=1$, $p=0.15$).

Fig. 2 Collection locations of New Hampshire (black), Missouri (dark gray), and Georgia (light gray) occur in three distinct climate zones. Days begin at May 1 and run through September 30. **a** New Hampshire has an overall cooler climate than Missouri or Georgia. Georgia is significantly warmer than either New Hampshire or Missouri (Kruskal–Wallis test $\chi^2 = 124.41$, $df = 2$, $p < 0.0001$). **b** Missouri has the most cumulative precipitation, while New Hampshire has the least amount of rainfall over the nesting season (Kruskal–Wallis test $\chi^2 = 2.24$, $df = 2$, $p = 0.29$)



We found a significant female bias in the first brood cell position in all three populations (NH: $\chi^2 = 19.27$, $df = 1$, $p < 0.0001$; MO: $\chi^2 = 8.32$, $df = 1$, $p = 0.001$; GA: $\chi^2 = 20.25$, $df = 1$, $p < 0.0001$; Table S1). In the first brood cell the percentage of female offspring in the New Hampshire population was 81.8%; Missouri was 100% and Georgia was 87.5%. In *C. strenua*, there was a female bias in the first brood cell position in both Missouri and Georgia (MO: $\chi^2 = 18.50$, $df = 1$, $p < 0.0001$; GA: $\chi^2 = 17.98$, $df = 1$, $p < 0.0001$; Table S2). The Missouri population had a higher percentage of females in the first brood cell position (94.7%), while Georgia had a smaller percentage (76.7%).

Offspring body size

Across all populations of *C. calcarata* daughters were significantly larger than sons (NH: Kruskal–Wallis test $\chi^2 = 66.64$, $df = 1$, $p < 0.0001$; MO: Kruskal–Wallis test $\chi^2 = 7.91$, $df = 1$, $p = 0.005$; GA: Kruskal–Wallis test $\chi^2 = 79.69$, $df = 1$, $p < 0.0001$; Table 1). Daughters and sons from the New Hampshire population were significantly larger than those from Missouri or Georgia (daughters: Kruskal–Wallis test $\chi^2 = 68.31$, $df = 1$, $p < 0.0001$; sons: Kruskal–Wallis test $\chi^2 = 60.32$, $df = 1$, $p < 0.0001$; Table 1). In the New Hampshire population, offspring

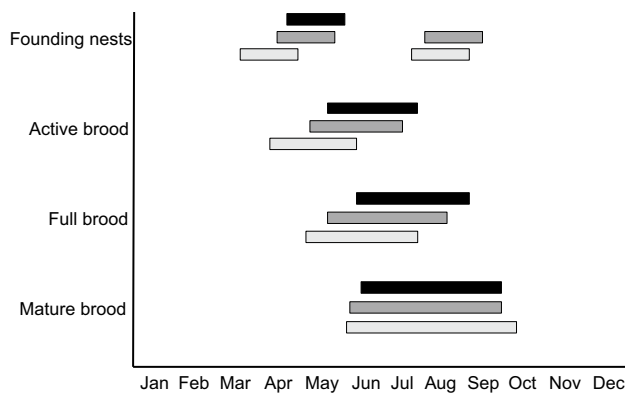


Fig. 3 Phenology of *C. calcarata* across the nesting cycle (May–September) in New Hampshire (black), Missouri (dark gray) and Georgia (light gray)

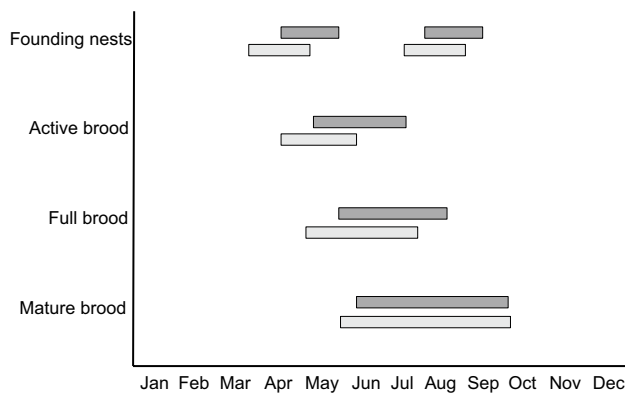


Fig. 4 Phenology of *C. strenua* across the nesting cycle (May–September) Missouri (dark gray) and Georgia (light gray). No *C. strenua* were collected in New Hampshire

size was not significantly correlated with brood cell position for daughters or sons (daughters: $R^2 = 0.01$, $F = 1.89$, $df = 1$, $p = 0.17$; sons: $R^2 = 0.005$, $F = 9.86$, $df = 1$, $p = 0.35$; Table S1A). In both Missouri (Linear regression: daughters: $R^2 = 0.08$, $F = 4.35$, $df = 1$, $p = 0.04$; sons: $R^2 = 0.03$,

$F = 1.83$, $df = 1$, $p = 0.18$; Table S1B) and Georgia (Linear regression: daughters: $R^2 = 0.05$, $F = 8.51$, $df = 1$, $p = 0.004$; sons: $R^2 = 0.004$, $F = 0.45$, $df = 1$, $p = 0.50$; Table S1C) daughters, but not sons, were significantly larger in later brood cells.

Overall, *C. strenua* daughters were significantly larger than sons (MO: Kruskal–Wallis test $\chi^2 = 69.11$, $df = 1$, $p < 0.0001$; GA: Kruskal–Wallis test $\chi^2 = 40.21$, $df = 1$, $p < 0.0001$; Table 1). Both daughters and sons from Missouri were significantly larger than those from Georgia (daughters: Kruskal–Wallis test $\chi^2 = 109.39$, $df = 1$, $p < 0.0001$; sons: Kruskal–Wallis test $\chi^2 = 94.83$, $df = 1$, $p < 0.0001$; Table 1). For daughters in Missouri, brood cell position was positively correlated with head width ($R^2 = 0.20$, $F = 31.44$, $df = 1$, $p < 0.0001$), but there was no correlation in sons ($R^2 = 0.01$, $F = 1.16$, $df = 1$, $p = 0.28$; Table S2A). There was no correlation between size and brood cell position in daughters or sons in Georgia (daughters: $R^2 = 0.007$, $F = 2.42$, $df = 1$, $p = 0.12$; sons: $R^2 = 0.0009$, $F = 0.23$, $df = 1$, $p = 0.64$; Table S2B).

Developmental rate

Although all brood were raised under the same laboratory conditions, the New Hampshire population of *C. calcarata* (37 ± 0.98 days) developed significantly faster than the Missouri (50 ± 1.17 days) or Georgia (57 ± 1.44 days) populations (Kruskal–Wallis test $\chi^2 = 30.90$, $df = 2$, $p < 0.0001$; Table S3). The total development time for the Missouri population was significantly shorter than for the Georgia population, resulting mainly from extended developmental time in the pupal stages. A similar pattern was seen in the developmental rates of *C. strenua*. The total development time was significantly longer in the Georgia populations (57 ± 1.45 days) compared to Missouri populations (53 ± 1.52 days) as seen by an extension in development times in pupal stages (Kruskal–Wallis test $\chi^2 = 25.80$, $df = 1$, $p < 0.0001$; Table S4).

Table 1 Summary of population demographics across three populations of *C. calcarata* and *C. strenua*

	<i>C. calcarata</i>			<i>C. strenua</i>	
	New Hampshire	Missouri	Georgia	Missouri	Georgia
Maternal HW ¹	2.07 ± 0.01 ^a	1.64 ± 0.04 ^b	1.77 ± 0.02 ^c	1.56 ± 0.02 ^d	1.45 ± 0.01 ^e
Clutch size ²	8.71 ± 0.31 ^a	12.46 ± 0.94 ^b	10.66 ± 0.66 ^b	10.79 ± 0.77 ^b	10.64 ± 0.37 ^b
Nest sex ratio ³	56 ^a	53 ^a	43 ^b	47 ^b	38 ^c
Daughter HW ¹	1.86 ± 0.01 ^a	1.66 ± 0.03 ^b	1.71 ± 0.01 ^c	1.52 ± 0.01 ^d	1.43 ± 0.01 ^e
Son HW ¹	1.66 ± 0.01 ^a	1.53 ± 0.03 ^b	1.54 ± 0.01 ^b	1.41 ± 0.01 ^c	1.35 ± 0.01 ^d

Significant p value differences are noted with superscript letters

¹Mean head width ± sem in millimeters reported

²Mean ± sem reported

³Percent male reported

Discussion

Variation in environmental conditions and ecological pressures across a latitudinal gradient has been shown to correlate with phenotypic variation in social behavior among populations in a variety of species (Danforth and Eickwort 1997; Wcislo 1997; Purcell 2011). To examine the role of environmental changes in the socially variable small carpenter bees, genus *Ceratina*, we compared life history traits between two populations of *C. calcarata* and three populations of *C. strenua* across their ranges. Southern populations of *C. calcarata* in Georgia have the longest active season, almost 7 months long compared to about 6 months in Missouri and only 5 months in New Hampshire (Fig. 3). Similarly, populations of *C. strenua* in the south have a month longer active season compared to populations in the north (Fig. 4). The previous research on *C. calcarata* populations in Missouri suggested bivoltinism in anecdotal reports (Rau 1928), but this was not observed in the previous studies of southern populations in Georgia (Kislow 1976). We found no evidence of bivoltinism in either species of *Ceratina*. We did observe a higher rate of individuals founding nests at the end of the season in southern populations, but these nests revealed no evidence of brood provisioning. We hypothesize that they were hibernacula.

In the southern populations of *C. calcarata*, the extended active season did not allow for the production of two separate broods, instead the mothers invested in larger clutches (Table 1). We found a positive relationship between clutch size and warmer climates with longer active seasons: Georgia 11 brood cells and 14 brood cells (Table 1, Kislow 1976, respectively), Missouri 12 brood cells (Table 1), Indiana 10 brood cells (Grothaus 1962; Johnson 1988), New Hampshire 9 brood cells (Table 1), and Ontario 7 brood cells (Rehan and Richards 2010a). In Georgia, we observed a strongly female-biased clutch sex ratio (Table 1). The previous research on *C. calcarata* has found males to be the smaller and, therefore, cheaper sex to produce, as less pollen provisioning is required to rear sons (Rehan and Richards 2010b; Lawson et al. 2016). An extended active season provides mothers in southern populations a prolonged foraging period which likely provides increased opportunities for investing in the costlier sex. Similar patterns have been observed in other bee species, where increased pollen availability results in more offspring of larger size (Minckley et al. 1994; Kim and Thorp 2001) and proportionately more offspring of the costlier sex (Peterson and Roitberg 2006).

In addition to increased clutch sizes and female-biased sex ratio, the warmer climates of the southern populations had significant effects on juvenile development. Our data on *C. calcarata* revealed a negative relationship between latitude and development time: Georgia 57 days, Missouri 51 days, and New Hampshire 46 days (Table S3), and a similar trend for *C. strenua*: Georgia 57 days, Missouri 53 days (Table S4). These results from our common lab rearing mirrored the development we observed when collecting from the field. The next step would be to test the effects of microclimate on development using translocation experiments. Similar experiments in *Exoneura robusta*, *E. nigrescens* (Cronin 2001) and *Lasioglossum calceatum* (Davison and Field 2018) have found effects of both habitat factors and genetic factors. This pattern has been observed in multiple ectotherms, where higher latitude environments have lower temperatures allowing for less time for growth and development (Tauber et al. 1986; Conover et al. 2009; Sniegula et al. 2011), and yet, populations at higher latitudes maintain similar life cycles to more southern populations (Corbet 2003). This indicates there is an optimal growth rate enforced by the environment and predicted by optimality models (Abrams et al. 1996). Metabolic rate likely influences development rates and has been shown to exhibit clinal variation in several arthropod and fish species (Wohlschlag 1960; Addo-Bediako et al. 2002; Williams et al. 2016). In addition, it is worth considering the effects of microclimates on developmental rate. For example, the previous research has demonstrated that in the northern populations of *C. calcarata* and *C. miqumaki*, bees laid in sun exposed nests took significantly longer to develop than those laid in shady sites (Vickruck and Richards 2012) implying nest preference also plays a role in development. Further research is needed to understand the physiological mechanisms and fitness costs and benefits associated with developmental and metabolic rates in this system.

Latitudinal gradients are associated with variation in many phenotypic traits. Bergmann's rule states that due to thermoregulatory constraints, individuals in colder climates will be larger than in warmer climates, resulting in a positive association between geographic range and body size (Bergmann 1847). Research across the animal kingdom supports this hypothesis (reviewed in Gaston and Blackburn 1996). Consistent with former studies, we found that both species of *Ceratina* follow Bergmann's rule. Individuals of *C. calcarata* collected in New Hampshire were significantly larger than individuals collected in southern, warmer regions. In addition, overall *C. strenua* were significantly smaller than *C. calcarata* and only occur in more temperate climates. This upper body limit of *C. strenua* may explain its limited range.

Social structure

Social polymorphism has been shown to occur across both altitudinal and latitudinal gradients in many bee groups, including the halictids (Plateaux-Quénu 2008) and allopapines (Cronin and Hirata 2003; Cronin and Schwarz 1999b). In one of our focal subsocial bee species, *C. calcarata*, the previous research has found evidence for two of the three hallmarks of eusociality: overlapping generations in the form of mother/offspring interactions and reproductive division of labor, the reproductive mother, and the worker dwarf eldest daughter. In light of the variation in social plasticity across a latitudinal gradient in other subsocial bee species with similar life histories, like the halictids and allopapines, we hypothesized that with the extended brood rearing season in the south, there could be the opportunity for the final hallmark of eusociality, cooperative brood care, if the mother produced two separate broods, and the dwarf eldest daughters helped to raise the second brood. Although we observed no evidence of bivoltinism in *C. calcarata* or *C. strenua*, we did find division of labor as evidenced by the presence of a dwarf eldest daughter in two new populations of *C. calcarata* and for the first time in its sister species, *C. strenua*. Dwarf eldest daughters are defined as females in the first brood cell position with a significantly smaller body size (Table 1); dwarf eldest daughters have been observed in *C. calcarata* (Rehan and Richards 2010b), *C. dupla* (Vickruck 2010), *C. flavipes* (Sakagami and Maeta 1977), *C. japonica* (Sakagami and Maeta 1984), and *C. okinawana* (Sakagami and Maeta 1995). The previous reports in *C. japonica*, *C. okinawana*, and *C. calcarata* have observed that dwarf eldest daughters forage as the worker for the nest, and feed siblings prior to overwintering (Sakagami and Maeta 1989; Rehan et al. 2014). Because the callow brood must be fed prior to overwintering, some studies have suggested that the dwarf eldest daughters provide assured fitness returns for the mother (Mikát et al. 2017; Shell and Rehan 2017). Because mothers have direct control of offspring sex and size (Lawson et al. 2016), the presence of dwarf eldest daughters is thought to be the product of maternal manipulation (Rehan and Richards 2010b; Shell and Rehan 2017). Variation in environmental conditions across the range of *C. calcarata* and *C. strenua* results in local adaptations including increased brood body size, investment in female brood, and plasticity in development time. Social structure of nests was stable across both species' range. The recurrence of dwarf eldest daughters across *Ceratina* species and across a variety of ecosystems implies an adaptive role for the production of worker daughters in otherwise subsocial bees.

Conclusions

By comparing populations across latitudinal gradients, we can compare the effects of extrinsic factors of the environmental gradient on physiological and group dynamics. These data sets can then be used to predict the effects of climate change on species and their distribution across the landscape. Here, we show that, following Bergmann's rule, body size of *Ceratina* species increased in response to colder climates. The smaller species, *C. strenua*, was not found in the colder climate of New Hampshire indicating a northern limit to the species distribution. The goal of comparative studies is to track common biogeographic patterns of group dynamics and identify ecological factors leading the evolution of more highly social behaviors. We found that the larger family groups of the small carpenter bee were found in more southern populations further supporting the role of increased temperatures and extended active seasons in facilitating more highly social groups.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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