

Climate Warming May Threaten Reproductive Diapause of a Highly Eusocial Bee

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ABSTRACT Climate changes are predicted to affect the diapause of many insect species around the world adversely. In this context, bees are of interest due to their pollination services. In southern Brazil, the highly eusocial bee species *Plebeia droryana* (Friese) (Hymenoptera: Apidae: Meliponini) exhibits reproductive diapause in response to the region's rigorous winters. That diapause is characterized by a temporary interruption in brood cell construction by nurse bees and egg-laying by the queen, regardless of other internal tasks underway in the nests. In this study, we evaluated whether *P. droryana* enter diapause under experimental conditions. *P. droryana* colonies were kept in a germination chamber, and the temperature was progressively reduced from 20°C over a period of a few weeks until diapause was detected. Additionally, we also estimated the environmental conditions in the actual geographic range occupied by *P. droryana* and modeled it for predicted changes in climate up to the year 2080. Our findings indicate that *P. droryana* enter diapause between 10 and 8°C. We also found that the current minimum winter temperature (10.1°C, median) in the distributional range of *P. droryana* will probably rise (13.4°C, median). Thus, if our experimental data are somewhat accurate, ~36% of the southern Brazilian *P. droryana* population may be active during the expected milder winter months in 2080. In this scenario, there may be a larger demand for pollen and nectar for that bee species. Greater conservation efforts will be required to preserve *P. droryana* populations and keep them viable in the coming decades.

KEY WORDS Apidae, beneficial arthropod, climate change, diapause, Hymenoptera

Climate change is predicted to adversely affect various animal behaviors, even those as strong and evolutionarily stable as adaptive behaviors and mutualistic interactions. For example, global warming may disrupt insect phenology, as well as mutualisms such as plant-herbivore, plant-disperser, and plant-pollinator relationships (Bale et al. 2002, Hegland et al. 2009, Warren and Bradford 2014). Likewise, behaviors such as diapauses, which are adopted by many insects to survive to unfavorable weather conditions, may cease due to climate change (reviewed by Bale and Hayward 2010). Over the next 50–100 yr some insect species that usually overwinter may be active during periods previously devoted to diapause due to the milder winters predicted to occur in coming decades (Fantinou and Kagkou 2000, Bale et al. 2002, Jepsen et al. 2008, Hegland et al. 2009, Tougou et al. 2009). In some cases, these behavioral changes could be disastrous because some of the insect species are migrants, invasive, or agricultural pests in crops such as corn, soybean, rice, and

cotton (Fantinou and Kagkou 2000, Bale et al. 2002, Jepsen et al. 2008, Hegland et al. 2009, Tougou et al. 2009).

For beneficial insects, such as pollinators (e.g., bees), which are important in global agriculture, the climate change has been already observed or predicted to modify its ecology and behavior. For example, bees may be displaced from areas of suitable habitat, resulting in reduced pollination success in certain local crops (Giannini et al. 2012). Likewise, shifts in plant phenology may disrupt the timing of bee–flower interactions and, consequently, restrict pollination (Hegland et al. 2009, Rafferty and Ives 2011, Scaven and Rafferty 2013). Climate change may also modify diapause in certain bees (e.g., *Bombus terrestris* L.), allowing them to become active in some regions where they usually overwinter (Stelzer et al. 2010, Owen et al. 2013).

Diapause is a very common behavior in insects, especially in temperate zones but also occurs in the tropics (Tauber and Tauber 1976). Diapause is a gradual and cyclic behavior in which insects temporarily cease developmental and behavioral activities to survive unfavorable environmental conditions (Tauber and Tauber 1976). Any disruption of diapause due to climate change is believed to imply profound effects on ecosystem stability (reviewed by Bale and Hayward 2010).

In the southern part of South America, some highly eusocial bee species (Apidae: Meliponini, stingless bees) are known to undergo diapause, possibly as an

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overwintering strategy to cope with the colder conditions of these subtropical regions. Briefly, during a nondiapauses period, there are complex and ritualized interactions between the nurse workers and the egg-laying queens (Sakagami 1982, Zucchi 1993). That is, the nurse workers build the brood cells, which are provisioned with larval food, receive one queen's egg, and finally are sealed by the nurse workers (Sakagami 1982, Zucchi 1993). This synchronized behavior between workers and queens in stingless bees is known as the provisioning and oviposition process, hereafter POP (Sakagami 1982, Zucchi 1993). But, during the colder months, POP is temporarily interrupted regardless of other ongoing tasks (such as forage, waste removal, building of wax pillars or involucre), in what has been called as reproductive diapause (reviewed by Santos et al. 2014).

Stingless bees are assumed not to actively thermoregulate their nests as honeybees do; that is; they do not use muscular contractions of their thoracic muscles to generate heat (Jones and Oldroyd 2006). Instead, many stingless bees build an involucre made of a wax and resin mixture (cerume) that passively thermoregulates the brood area, isolating both brood combs and the adult nest population from temperature fluctuations as much as possible (Jones and Oldroyd 2006). In fact, many stingless bee species that perform reproductive diapause build plentiful wax pillars or layers and perform at a rather slow or lethargic pace during the peak of winter in Brazil (June and July) without any apparent active thermoregulation (reviewed by Santos et al. 2014).

The main stingless bees species observed to exhibit reproductive diapause belong to the genus *Plebeia* Schwarz, including *P. droryana* (Friese), *P. emerina* (Friese), *P. wittmanni* Moure & Camargo (Friese), *P. nigriceps* (Friese), *P. remota* (Holmberg), *P. julianii* Moure, and *P. saiqui* (Friese) (reviewed by Santos et al. 2014). Many of these bees are important crop visitors and pollinators of coffee, cucumber, strawberry, oilseed rape, orange, tomato, and other plants in tropical and subtropical regions (Heard 1999, Slaa et al. 2006). Thus, those crops, or even wild plants, may experience some deficit or improvement in its pollination services because of an outbreak of diapause in any stingless bee species during winter.

Thus, our main goal was to predict whether *P. droryana* populations will exhibit reproductive diapause in the future, given that its area of distribution will probably suffer an increase in ambient temperature from climate changes in the near future. To this end, reproductive diapause in *P. droryana* was experimentally induced in the laboratory to investigate the threshold temperature triggering diapause. To place these results into the context of likely climate change, we investigated the environmental conditions within the current geographic distribution of *P. droryana* and modeled entrance to diapause on the basis of likely climate change effects in the future (to the year 2080). Next, we analyzed our data from the experimental induction of diapause for this bee species and compared the results with the future temperature predictions for the geographical range of *P. droryana*.

Materials and Methods

Colonies. Seven *P. droryana* colonies, termed A to G, were standardized for size (17 by 17 by 12 cm³). Every colony initially had a single egg-laying queen, ~100 workers, and brood combs with ~500 workers ready to emerge. No literature data exist concerning the emergence rate in this bee species, but from 10 to 30 new workers were expected to emerge daily (personal observation). Three honey and pollen pots from *P. droryana* were provided from the summer of December 2013 to February 2014. In preparation of the diapause induction experiments, these colonies were kept for about 2 wk at the stingless bee apiary of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), in Porto Alegre, Brazil (30° 1'58" S, 51° 13'48" W). Bees from the colonies were allowed to fly and forage freely during this time.

Following this establishment period, the colonies were transferred to a biochemical oxygen demand (BOD) incubator (model Luca-161/04) with controlled relative humidity (RH 70%) at a pre-experimental temperature of 25°C and in constant darkness (a photoperiod of 0:24 [L:D] h). At this time, all colonies were constructing brood cells (13.59 ± 9.57 [mean ± SD] per POP cycle), and the queens were laying eggs.

Diapause Induction Experiment. We commenced the experimental induction of the reproductive diapause 1–2 wk after introduction of the nests into the germination chamber under the constant conditions described above. To achieve experimental results, we initially decreased the temperature from 25 to 20°C, followed by a further decrease of 2°C every three days until diapause was detected. Data loggers (model U23-001 HOBO Pro v2) were installed inside the germination chamber and within the hives as well (near the brood combs) to compare temperatures inside and outside of the nests.

During the gradual reduction in temperature, we observed overall workers' activities in harvesting food and manipulating waste. However, our focal observation was to quantify the number of brood cells built by the nurse bees and the number of eggs oviposited by the queens. Observations were conducted three times every day (at 0800, 1300, and 1800 hours) throughout the entire experiment. These observations were made individually on every colony for 2–3 min under a red light in a darkroom with no climate control. Colonies were fed daily at 0800 hours with 5 ml of honey syrup (1:1, honey:water) and 0.50 g honeybee's pollen. In addition, we noted the presence or absence and fullness of wax involucre covering the brood comb.

We assumed reproductive diapause had been successfully induced and noted the associated temperature when bees ceased to build brood cells and when the queens ceased egg-laying, regardless of other internal colony activities, such as the manipulating of syrup or pollen, waste, and wax (see Results). One week after diapause had been induced, we returned the temperature in the BOD incubator to 25°C again to observe whether, and when, the colonies would cease diapause.

Data Analysis. The data were analyzed using statistical and graphical packages in the R program (R Core Team 2014) and carried out in the RStudio environment (RStudio Team 2012). First, a second-order polynomial (quadratic) regression analysis was performed using ordinary least squares and assuming fixed x values to find the line that minimizes the square errors in the y values. The analysis of the relationship between temperature and the number of brood cells built was performed by adapting the quadratic model in the `lm` function of the `stats` package (R Core Team 2014).

In addition, we compared the temperature inside the colonies with the external temperature (in the BOB incubator) to investigate whether *P. droryana* would be able to thermoregulate somewhat in their nests. This analysis was carried out employing the Kruskal–Wallis test using the R `stats` package (R Core Team 2014). After getting results from the Kruskal–Wallis test, we applied the adjusted Dunn's test using the Holm–Sidak model for a pairwise comparison of the likely differences in temperature (median) between the BOD incubator and the diapausal colonies using the `dunn.test` package (Dinno 2014). Both analyses were visualized using the `ggplot2` package (Wickham 2009).

Modeling. In the first step, we used Species Distribution Modeling (also called Habitat Suitability Modeling) to detect the most suitable habitats for *P. droryana* under current climate conditions. Subsequently, we used Geographic Information System methodology to detect the level of temperature change in suitable habitats for *P. droryana* from current climate conditions, projecting into the near future and until the year 2080, to detect the potential impacts of climate change on the diapause behavior of this species. These steps are described below.

Modeling Framework

Environmental Variables. We obtained 20 environmental layers of topoclimatic data from Worldclim (Hijmans et al. 2005) with a spatial resolution of 5 min of arc (cell size ~ 10 km), worldwide (with the exception of latitudes south of 60°). Nineteen layers showed annual trends of seasonality, extremes and averages of temperature and precipitation for the period of 50 yr (1950–2000), and one layer showed altitude. Thereafter, all layers were clipped to a square area (4,400 by 4,400 km^2) covering territorial Brazil, with the exception of some Brazilian islands farthest to the east. For six temperature variables—annual mean temperature, minimum temperature, maximum temperature, temperature annual range, mean temperature of warmest quarter, and mean temperature of coldest quarter—we obtained future scenarios estimating climate change up to the year 2080 at the same resolution as the current data. The climate change layer projections were obtained from the Goddard Institute for Space Studies—NASA (GISS-NASA, 2014)—at the higher values for Representative Concentration Pathways: RCP 8.5 (AR5-IPCC, 2013).

Native Presences and Pseudoabsences of *P. droryana*. We used presence-only records for *P. droryana* collected from two main sources: 1) collections and museums surveyed via internet biodiversity

databases, i.e., SpeciesLink (<http://splink.cria.org.br/>), our own database at the Museum of Science and Technology of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), and by correspondence with experts from other Brazilian institutions (see Acknowledgments); and 2) data extracted from the published literature, i.e., Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region—online version (Camargo and Pedro 2013). Duplicate records were filtered out of the dataset so that two or more records with the same geographic coordinates were excluded, leaving only exclusive species geolocations. Our final dataset comprised 96 exclusive location records of *P. droryana* (minimum distance between points = ~ 333 m; maximum distance between points = $\sim 3,412$ km).

Nothing is known about the locations from which *P. droryana* is truly absent. We therefore generated five random pseudoabsence datasets (PA1 to PA5) without replacement (i.e., with no repeated locations between them). Each PA dataset contained 10 times the number of records compared with the known presence dataset and therefore contained 960 distinct locations where *P. droryana* may potentially be absent. The generation of random points was defined as to never create points that coincided spatially with true presence locations.

Modeling procedure. The *P. droryana* presence dataset was randomly divided to provide 75% of the records to train the models while the remaining (25%) were used to mathematically evaluate the models using the True Skill Statistic (Allouche et al. 2006). This random division was repeated five times (RUN1 to RUN5) to obtain a robust estimate for the algorithm's performance (Franklin 2009).

We used the `Biomod2` package version 3.1.25 (Thuiller et al. 2009) for R (R Core Team 2014) to develop the species distribution models with 10 commonly applied algorithms for a wide range of species (Phillips et al. 2006, Franklin 2009, Thuiller et al. 2009). Each algorithm uses its own mathematically based analysis logic, but the algorithms group into two main types of mathematical methods: Regression and Machine Learning Analysis. The algorithms selected were Artificial Neural Networks (ANN; Ripley 1996), Generalized Boosted Models (GBM; Ridgeway 1999, Friedman 2001), Random Forests (RF; Breiman 2001), Maximum Entropy (MAX; Phillips et al. 2006), Surface Range Envelopes (SRE; Busby 1991), Generalized Additive Models (GAM; Hastie and Tibshirani 1990), Generalized Linear Models (GLM; McCullagh and Nelder 1989), Multivariate Adaptive Regression Splines REGRESSÃO (MARS; Friedman 1991), Classification Tree Analysis (CTA; Breiman et al. 1984), and Flexible Discriminant Analysis (FDA; Hastie et al. 1994).

We used the standardized parameters in `Biomod2` default settings (Thuiller et al. 2009, 2013) and `Maxent` (Phillips and Dudík 2008), aiming to maintain metric standardization among sequential rounds of modeling with different algorithms; these packages allowed us to share the same datasets, general parameters, and evaluation methods in a way that provided comparability of the predictive quality among models.

The maximum possible combinations of five pseudoabsence datasets (PAs), five partitions of presence data (RUNs), and 10 different algorithms (ALGO1 to ALGO10) generated 250 models, which estimated suitable and unsuitable habitats for *P. droryana* in the various environmental layers. To reduce the number of model rounds and select the set of most accurate models, we evaluated each model quality using True Skill Statistics (TSS). Only the models that reached TSS values higher than 0.75 were retained. TSS is a synthetic index that considers the ability of each model to correctly predict suitability in places where there are true presences (sensitivity) and unsuitability where there are absences (specificity)—or pseudoabsences in this case—and is not sensitive to prevalence (Allouche et al. 2006). TSS values normally range between 0 and 1, but in some cases, TSS values could be negative; zero proximity and negative values indicate that the model is not better than a random result, i.e., useless to detect habitat suitability (Jiménez-Valverde and Lobo 2007, Jones et al. 2010).

For the method developed here, we needed models with a clear dichotomy between suitable and unsuitable habitats for *P. droryana*; to this end, we defined the thresholds that maximized both the sensitivity and specificity of each modeling round, respectively. Next, every model was converted into binary values (1 for suitable and 0 for unsuitable habitats). This threshold parameter has been believed to provide the most accurate predictions (Jiménez-Valverde and Lobo 2007, Gallien et al. 2012).

Ensemble Forecast and Model Projection. After selecting the most accurate set of binary models, we combined them into a unique model using the Committee Averaging Method of Ensemble Forecasting Model using the integrated function of the Biomod2 package (for further details see Thuiller et al., 2009). The main advantage of the ensemble forecast model is that it provides a lower mean error than any of its individual constituents (model rounds, in this case), and therefore improves predictive accuracy (Gallien et al. 2012). The ensemble model also provides analytical simplification through the combination of multiple models into a unique, more robust, and precise model.

We projected the ensemble forecast model using the software of Geographic Information Systems ArcGIS (ESRI 2010) and converted the grid cells raster with binary values 1 (suitable habitats) to georeferenced feature points. Subsequently, we used the feature point datasets created to capture the environmental information for the six temperature layers selected for the current scenario, as well as the respective ones for the future scenario. In this way, we generated a dataset containing variation in these temperature variables in the range of suitable *P. droryana* habitats for current and future scenarios. This dataset was thereafter referred to as the Thermal Variations on Suitable Habitat dataset (TVSH).

Average Minimum Temperature Measures. Finally, we also compared the average minimum temperature measures in the range of suitable *P. droryana*

habitats in current and future scenarios using Wilcoxon signed rank paired test, which was carried out in the *stats* package (R Core Team 2014).

Results

Temperature Threshold for Reproductive Diapause. The relative humidity inside the colonies was $94.19 \pm 4.2\%$ (mean \pm SD), while the relative humidity within the germination chamber was $70 \pm 0.5\%$ (mean \pm SD). A Kruskal–Wallis test indicated significant differences between the temperatures in the colonies and those in the BOD incubator ($\chi^2=19.239$, $df=7$; $P<0.01$). After re-analyzing these data using Dunn's test with the Holm–Sidak method, it was possible to verify that a significant difference occurred between the temperature in the BOD and the temperatures in colonies A and C (Fig. 1).

The quadratic polynomial regression demonstrated a significant correlation between temperature and brood cell building rate ($F=170.2$, $df=438$, $r^2=0.43$, $P<0.0001$; Fig. 2). The residual mean-square error was 5.264 with 438 degrees of freedom. A quadratic function was found ($y = \beta_0 + \beta_1x + \beta_2x^2 + e$), where y is the dependent variable (number of brood cells), β_0 is the intercept parameter, β_1 is the linear effect parameter, β_2 is the quadratic effect parameter, x is the independent variable (temperature), and e is the related random error. We therefore verified that brood cell building could continue between 9 and 10°C, albeit at very low rates (0.97 to 2.51 brood cells per POP).

Worker and Queen Activities. During the early weeks, while temperatures were progressively decreasing from 20 to 14°C, *P. droryana* workers actively worked on combs and built many brood cells (10.73 ± 6.93 [mean \pm SD]). During each cycle of the POP, each one received an egg laid by the queen soon after it was ready (Supp Video 1 [online only]). Furthermore, workers actively collected the food offered each day and stored it. Almost no wax involucre were built on the brood combs. Nevertheless, as the temperature was gradually decreased, the POP also began to decrease regardless of the daily food input. Between 16 and 12°C, pollen was collected more rarely. Furthermore, many workers (± 15 to 30) started building wax involucre, and these became progressively larger, gradually covering all of the brood combs. Other tasks, such as the manipulation of waste and the storage of food (sugar syrup), continued although fewer workers were involved in these tasks.

At temperatures above 12°C in the germination chamber, three daily cycles of POP were usually observed, one in each observation period in each day. At 12°C and lower temperatures, it took longer for brood cells to be built. Building took almost all day, and the same cells were still being built during subsequent observations. In all colonies, we observed that queens rarely visited brood combs, and consequently, almost no eggs were laid. At 10°C, in two colonies (E, G), workers started to build a single cell and three cells, respectively, but the cells remained unfinished

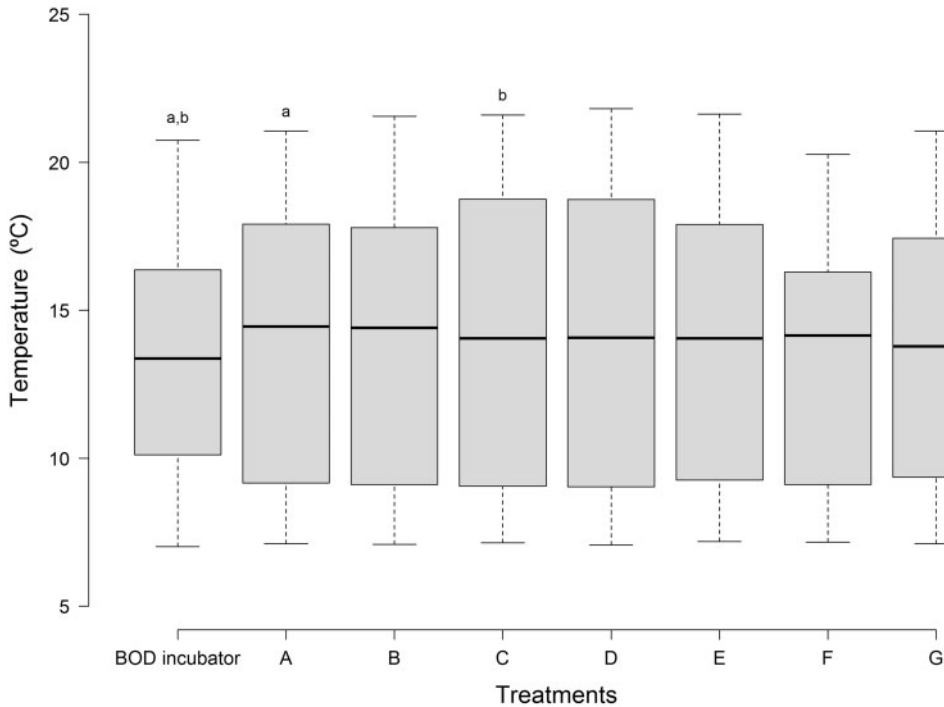


Fig. 1. A comparison of temperatures measured in the BOD incubator with those measured inside colonies (A through G) over the course of the experiment for *P. droryana* (Hymenoptera: Apidae). Boxplot: median, first and third quartiles, upper and lower lines indicate the maximum and minimum values.

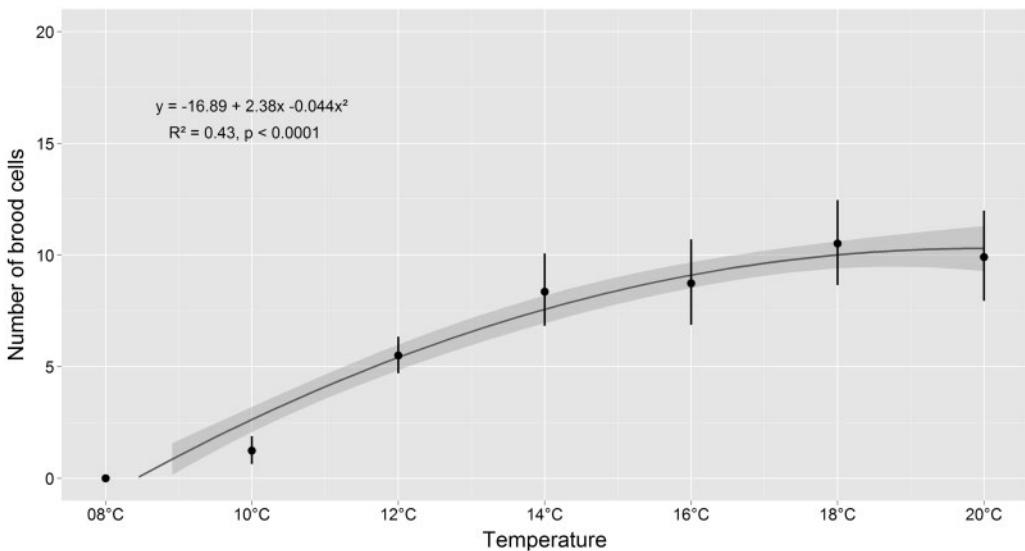


Fig. 2. The number of brood cells constructed in *P. droryana* (Hymenoptera: Apidae) colonies per provisioning oviposition process at various temperatures. Quadratic regression: black points—means, shaded—standard errors, vertical lines—99% confidence interval, dark gray line—second order polynomial line.

and no eggs were laid. These two colonies were therefore considered to have entered reproductive diapause. At 8°C, the remaining colonies ceased brood cell construction and were also considered to have entered reproductive diapause.

One week after all colonies had entered reproductive diapause, the colonies were exposed to 25°C. In the first 3d following this increase in temperature, we observed activity in many workers and the queens inside the nests, but without POPs. After 3d, the

workers from three colonies started building brood cells again, and eggs were laid in them by the queen. On the fourth day, another *P. droryana* colony also recommenced POP. On the sixth day, two more *P. droryana* colonies terminated diapause. Finally, on the eighth day, the last colony terminated reproductive diapause. The number of brood cells built after the cessation of reproductive diapause was, on average, 7.85 ± 3.38 (mean \pm SD) cells per POP.

Suitable Habitats and Temperatures for *P. droryana*. The areas of suitable habitat detected by the modeling procedure suggested that *P. droryana* is a species more suited to the current climate of the Atlantic Forest domain; indeed, $\sim 67\%$ of suitable habitat cells were located in this Brazilian biome (Fig. 3). An affinity to the marginal areas of the Cerrado Biome (Brazilian savanna) was also detected under current climate conditions; that is, $\sim 25\%$ of the suitable habitat cells detected were located in the Cerrado.

Among the six thermal features analyzed (Supp Figs. 3–5 [online only]), only the minimal temperature layer was shown to be a potentially determining environmental factor; that is, a factor that could induce or suppress diapause depending on the threshold in specific areas. Based on the area of climatically suitable habitats mapped for *P. droryana*, we measured the variation in minimum critical temperatures against the actual climate conditions and the estimated shifts in future climate conditions. Overall, we estimated that within the range of *P. droryana*, whose minimum temperature is now 10.1°C (median), the minimum temperature will probably be higher (13.4°C , median) in the year 2080 (V_{4100} , $P < 0.0001$; Fig. 4).

Based on the minimum critical temperature necessary to experimentally induce diapause in *P. droryana*, we show that there is a high likelihood that *P. droryana* will need to enter diapause in regions where the temperature is less than or equal to than 8°C (blue areas in Fig. 5A and B). On the other hand, in regions where the lowest temperatures in winters are above 10°C , *P. droryana* probably will not undergo diapause (orange areas in Fig. 5A and B). Finally, there also appear to be mixed or “transition” areas, in which low temperatures in the colder months may fluctuate between 8 and 10°C . In those areas, certain *P. droryana* colonies, predictably, may or may not enter diapause (green areas in Fig. 5A and B).

Therefore, a future scenario for the year 2080 is that there will be a 35.9% increase in the actual *P. droryana* population that may avert diapause due to milder winter months (Figs. 5B and C, and 6). Of the 35.9, 23.89% will be in the mixed zones (where diapause may or may not occur) and the remaining 12% will be in areas where diapause likely will not occur (Figs. 5B and C, and 6).

Discussion

Experimental Induction of Reproductive Diapause. Our findings demonstrate that even during a time not devoted to reproductive diapause, it is possible to experimentally trigger this behavior in

P. droryana by progressively decreasing the temperature over the course of a few weeks under laboratory conditions. We initially observed that these bees gradually decrease the rate of brood cell building as the temperature progressively declines until full reproductive diapause is achieved. The worker bees respond somewhat progressively, decreasing brood-cell building, while gradually increasing the amount of wax involucres being built over the brood combs.

A thick, multilayered involucre that surrounds the brood combs to ameliorate cold microclimatic conditions seems to be very common in *P. droryana* (Drumond et al. 1996). The building of wax involucres seems to help in passive protection of the brood from colder temperatures, thereby helping somewhat in the thermoregulation of nests prior to the onset of diapause. However, the use of involucres to thermoregulate does not seem to be so effective in the incubators where the temperatures in the *P. droryana* colonies tended to be similar to the temperatures in the BOD incubator. Only two colonies exhibited temperatures different from the temperature of the BOD incubator, but even for those colonies, the difference was not enough to avert diapause in the colonies.

We observed that the threshold temperature for inducing diapause in *P. droryana* fluctuated between 10 and 8°C . It is known that under natural conditions *P. droryana* and other stingless bee species enter diapause before such a low temperature is reached (reviewed by Santos et al. 2014). But, below 8°C , most stingless bee species under natural conditions are already in diapause (reviewed by Santos et al. 2014). Thus, the fact that our colonies entered diapause at such low temperatures (8 to 10°C) may be due to an absence of other environment cues that are used by these bees to synchronize and begin diapause. Nevertheless, other studies are needed to determine the role of other environmental cues.

We also noticed that when the diapausing *P. droryana* colonies were treated with the drastically warmer temperature (25°C), the colonies varied in their termination of diapause, which happened over a few days before the colonies returned to usual behavior. This behavior may suggest a weakness in the experiment, or it may suggest that real diapause was not induced. However, we believe we were successful because some tasks continued, for example, wax, food, and waste manipulation, though slowly, in the nests during the diapause. In other words, only POP was arrested, not other tasks. Furthermore, the termination of diapause in as few as 2–3 d once the weather becomes milder may occur in a natural way even with colonies that have been in diapause for several months, rather than few weeks, as observed for *P. remota* (Ribeiro et al. 2003), a stingless bee species that is closely related to and overlap itself to distributional range of *P. droryana* (Camargo and Pedro 2013).

Reproductive Diapause and Climate Warming. If our experimental data are relevant to our future temperature estimates, then it is possible to predict that part of the *P. droryana* population in southern Brazil ($\sim 36\%$) will respond to the climate

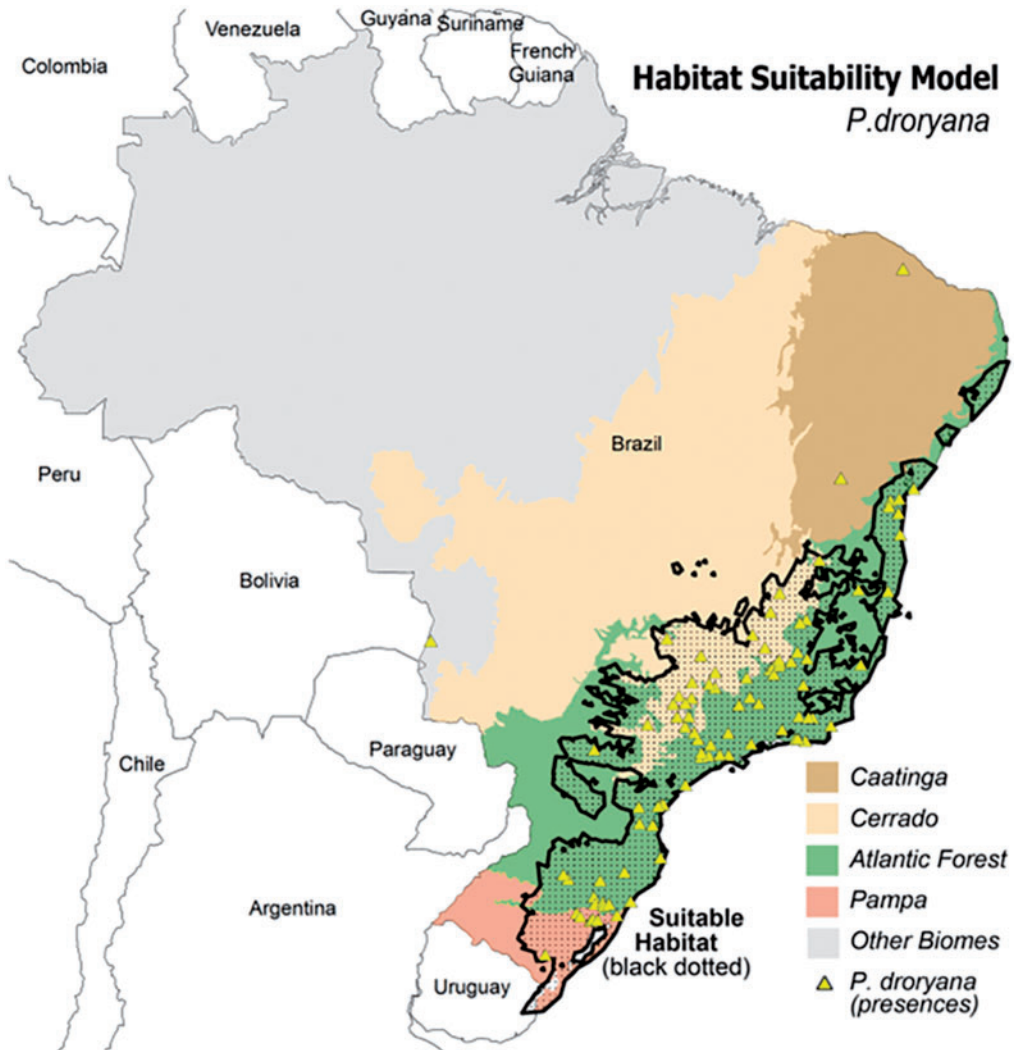


Fig. 3. Areas of suitable habitat (black dotted) for *P. droryana* (Hymenoptera: Apidae) in current climate conditions.

warming occurring between now and the year 2080 by cessation of reproductive diapause. Of this percentage, ~24% will inhabit “transition” or mixed regions where the average minimum temperature in winter will be between 8 and 10°C. Thus, it is possible that under such conditions, egg-laying rates may be very low (0.97–2.51 brood cells per POP) in some *P. droryana* colonies.

Although our experimental data suggest a strong possibility that certain *P. droryana* populations may undergo alterations in their capability to enter diapause in southern Brazil, according to our climatic modeling, such an effect will indeed occur in coming decades. However, concrete alterations in the diapause behavior of some natural insect populations have been confirmed already because of, among other things, increasing temperatures (Vaz Nunes and Saunders 1989, Fantinou and Kagkou 2000, Tougou et al. 2009). In other words, researchers have described moth,

and bug populations that are spending less time in diapause or, in contrast, spending more time in diapause due to milder temperatures in certain temperate regions (Vaz Nunes and Saunders 1989, Fantinou and Kagkou 2000, Tougou et al. 2009).

Similarly, certain *Bombus terrestris* bumblebee populations are experiencing disruptions in diapause, supposedly due to milder weather in the Northern Hemisphere (Stelzer et al. 2010, Owen et al. 2013). The colonies of these pollinator insects are staying active throughout years, in some cases, and the nest-founding queens are continuing to forage and oviposit during temperate winter, a time usually devoted to diapause (Stelzer et al. 2010, Owen et al. 2013). The researchers believe that successive generations of *B. terrestris* may be adapting to climate warming by subtly modifying their phenology and, consequently, averting diapause in these regions (Stelzer et al. 2010, Owen et al. 2013). Whether this phenomenon may also be

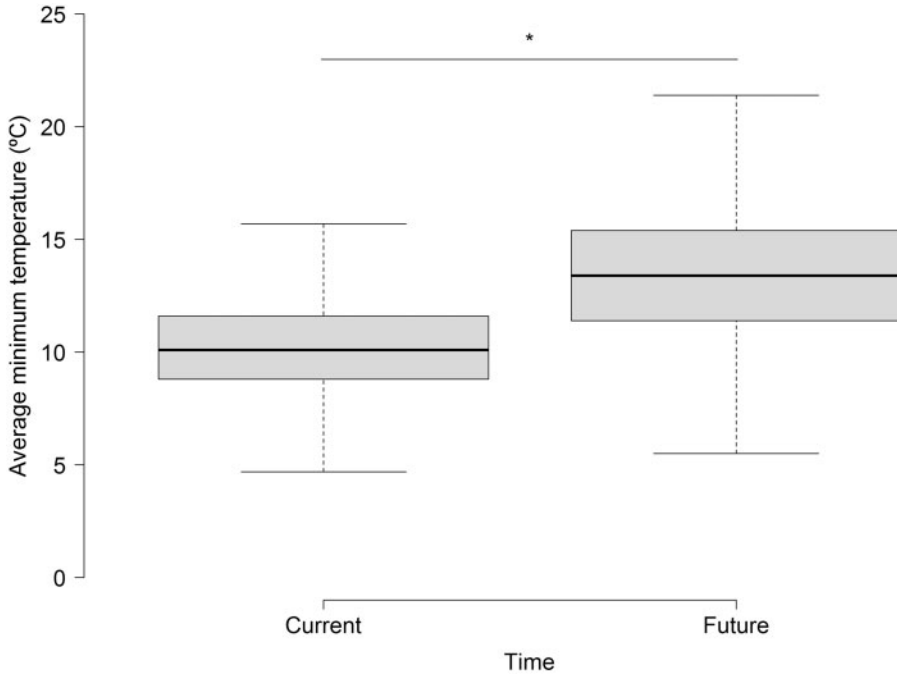


Fig. 4. A comparison of the average minimum temperatures in current versus future (2080) scenarios under a habitat suitability model of *P. droryana* (Hymenoptera: Apidae) in southern Brazil. Asterisks indicate significant differences (< 0.01). Boxplot: median, first and third quartiles, upper and lower lines indicate the maximum and minimum values.

Diapause Change based on Minimum Temperature in Suitable Habitat for *P. droryana*

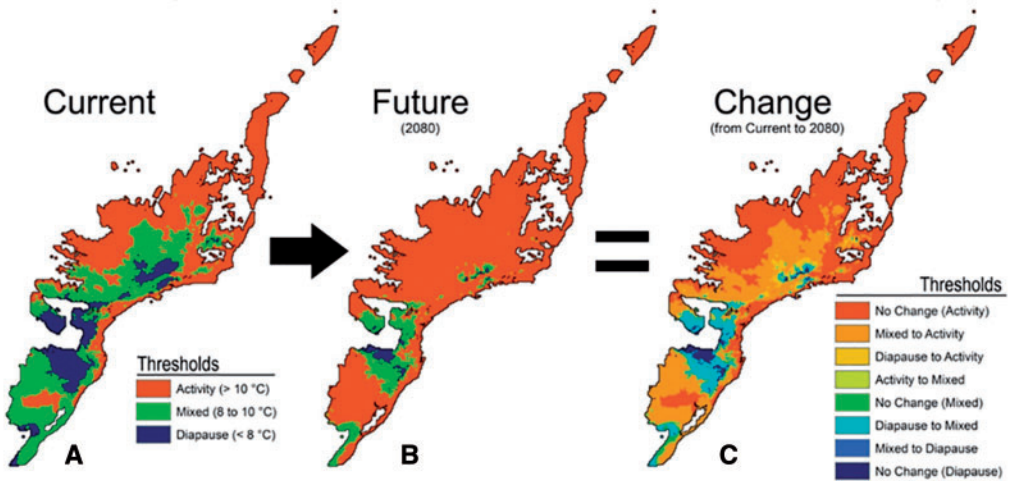


Fig. 5. Geographic distribution of *P. droryana* (Hymenoptera: Apidae) in Brazil under a habitat suitability model. The images (A—actual, B—future [2080], C—expected change from A to B) due to an increase in minimum temperatures in the colder months during coming decades. Black, blue: Areas where natural populations of this bee species will, perhaps, continue to exhibit reproductive diapause; Green: Mixed zones (or “transition” areas) where we predict that some populations will exhibit reproductive diapause, while others will show no reproductive diapause; Orange: Zones where populations will cease reproductive diapause.

directly applicable to the stingless bee *P. droryana* is still undetermined; however, our data (from the experimental diapause induction and from the climatic modeling) support this possibility.

Nevertheless, care is needed because one must be aware that the behavioral plasticity or adaptation of any insect to any selective pressures may be much more complex than our results can actually show by

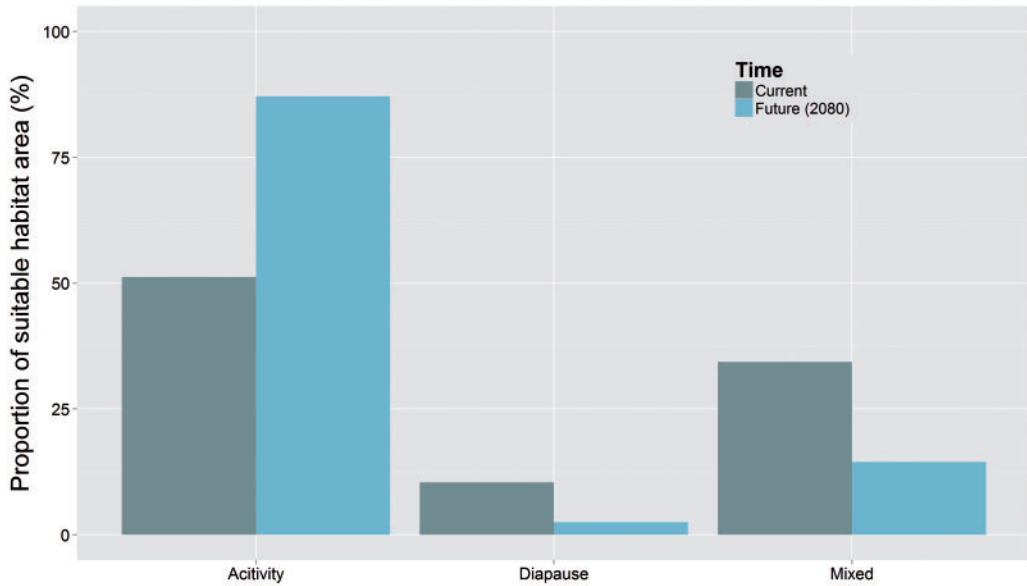


Fig. 6. Proportion of suitable habitat area for *P. droryana* (Hymenoptera: Apidae) populations that currently perform or do not perform reproductive diapause (dark blue) and model results for these two behaviors for the year 2080, considering likely climate change (light blue).

controlling a single environmental variable (temperature). Even more care is required when these results come from experiments performed in the laboratory rather than under field conditions. Thus, although our findings indicate that a portion of *P. droryana* population will be affected by warmer temperatures in terms of its diapause behavior by the year 2080, we do not know at this time whether other environment variables also will interfere in this process. Therefore, any conclusion that does not take other variables into account would be largely speculative.

In summary, global warming may affect the life cycles of beneficial insects, such as pollinators, in different ways over the coming decades. Thus, future modifications in plant–pollinator interactions (Hegland et al. 2009, Rafferty and Ives 2011, Scaven and Rafferty 2013), geographical ranges of many species (Giannini et al. 2012), and even alterations in diapause pattern due to climate changes (Bale and Hayward 2010, Stelzer et al. 2010, Owen et al. 2013, Scaven and Rafferty 2013) are predicted. Therefore, if our scenario modeled for *P. droryana* is accurate, there may be a larger future demand for floral resources (pollen and nectar) for this bee species to provide food for its larvae and energy reserves for adults during less rigorous winters in southern Brazil. Greater conservation efforts will be required to maintain suitable areas for nesting, foraging, and sheltering to sustain *P. droryana* populations in coming decades.

Supplementary Data

Supplementary data are available at *Economic Entomology* online.

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