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Oyamel fir forest trunks provide thermal advantages for overwintering monarch butterflies in Mexico

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Abstract. 1. Survival of overwintering monarch butterflies following severe wet winter storms in Mexico is substantially higher for butterflies that form clusters on the oyamel fir tree trunks than for those that form clusters on the fir boughs.

2. Thermal measurements taken at similar elevations with a weather station on the Sierra Chincua and within a Cerro Pelon and a Sierra Chincua overwintering area indicated that clustering on the fir trunks provides dual microclimatic benefits for the butterflies.

a. At night, the minimum surface temperatures of all firs combined averaged 1.4 °C warmer than ambient forest temperatures, thereby enhancing protection against freezing for monarchs that are either wet or dry. We term this the 'hot water bottle effect.'

b. During the day, the maximum surface temperatures of all firs combined averaged 1.2 °C cooler than ambient, a difference sufficient to lower the loss of the butterflies' lipid stores over the 154-day wintering season.

3. Larger diameter trees increase both microclimate benefits.

4. The results add a new dimension to improving the conservation management guidelines for the Monarch Butterfly Biosphere Reserve. Strict enforcement against culling of larger trees and in favour of promoting old-growth oyamel forests will enhance two microclimatic benefits: butterfly mortality during severe winter weather will be reduced, and the butterflies' lipid savings over the winter will be enhanced.

Key words. Conservation, hot water bottle effect, large and small trees, microclimate, Monarch Butterfly Biosphere Reserve management, thermal buffering, thermal inertia.

Los troncos del bosque de oyamel proporcionan ventajas térmicas para las mariposas monarca invernantes en México

Resumen: 1. Después de las tormentas invernales, la posibilidad de sobrevivencia para las mariposas monarca invernantes es considerablemente mayor para aquellas que se agrupan alrededor de los troncos de los árboles que para las que se agrupan en las ramas.

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2. Tomamos registros de temperatura mediante una estación de meteorológica que instalamos en Sierra Chincua, varios termómetros instalados dentro de las áreas de invernación de Sierra Chincua y Cerro Pelón. Los resultados indicaron que el agrupamiento en los troncos del arbolado provee doble beneficio microclimático para las mariposas.

a. Durante la noche, la temperatura de la superficie de los troncos de oyamel es en promedio 1.4 °C más cálida que la temperatura ambiente del bosque. De tal manera, los troncos incrementan la protección contra el congelamiento, tanto para mariposas húmedas como secas. Llamamos a lo anterior como 'efecto de la botella de agua caliente.'

b. Durante el día la temperatura de la superficie de los troncos de oyamel es en promedio 1.2 °C más fresca que la ambiental, lo cual representa una diferencia significativa para reducir la pérdida de reservas de lípidos de las mariposas durante los 154 días del periodo de invernación.

3. Los árboles con diámetros más grandes incrementan ambos tipos de beneficios microclimáticos.

4. Los resultados añaden una nueva dimensión para mejorar los lineamientos del plan de conservación de la Reserva de la Biosfera Mariposa Monarca. Una estricta aplicación de la ley contra la extracción de los árboles más grandes y a favor de promover los bosques maduros de oyamel incrementaría dos beneficios microclimáticos: se reduciría la mortalidad de mariposas en eventos climáticos invernales severos; y, se incrementaría el ahorro de lípidos a lo largo del invierno.

Palabras clave: Conservación, árboles grandes y pequeños, efecto de la botella de agua caliente, microclima, Manejo de la Reserva de la Biosfera Mariposa Monarca, protección térmica, inercia térmica.

Introduction

In their overwintering forests in Mexico, monarch butterflies (*Danaus plexippus* L., Lepidoptera: Nymphalidae, Danainae) form colonies with dense clusters on both the boughs (Fig. 1a) and trunk (Fig. 1b) of coniferous trees, especially the oyamel fir (*Abies religiosa* H.B.K., Pinaceae), but also smooth bark Mexican pine (*Pinus pseudostrobus* Lindl., Pinaceae) and Mexican cedar (*Cupressus lusitanica* Miller, Cupressaceae, synonym of *C. lindleyi*; Anon, 2007). In a 2.7 ha Sierra Chincua colony in the state of Michoacan during the 1978–1979 overwintering season, Calvert (2004) estimated that the colony included 1026 oyamel trees, of which 16% had clusters on their boughs and 37% on their trunks. Even though twice the number of oyamel trees had trunk clusters, because of the large number of boughs per tree, Calvert estimated that about 90% of the monarchs were clustering on the boughs. This preponderance of monarchs in bough clusters has been observed almost every year since January 1976 (Brower, 1977; Brower *et al*. 1977; Brower, field notes) and led us to speculate that overall survival rates would be higher in the bough clusters. Contrary to this prediction, Calvert *et al*. (1983) determined that monarchs clustering on tree trunks in another Sierra Chincua colony during January, 1981, had substantially higher survival rates following a prolonged period with rain, hail, and snow.

Why should survival be higher on tree trunks? Because of the high heat capacity resulting from their large mass, tree trunks respond slowly to surrounding environmental temperatures. Both theoretical and empirical data in Derby & Gates (1966), Bakken & Gates (1975), Gates (1980), and Geiger *et al*. (2003) suggest that thermal buffering may provide increased microclimatic advantages for overwintering monarchs clustering on tree trunks compared to monarchs clustering on the tree boughs which are more strongly affected by convective heat exchange due to wind (Tibbals *et al*., 1964; Chown & Nicolson, 2004). In a preliminary study, Lear (1998) found that trunk temperatures of pine trees in Virginia during December were warmer than ambient at night and cooler than ambient during the day, and that the moderation was greater on larger diameter trees.

To investigate the trunk microclimate hypothesis, this paper ties together several studies in the overwintering area that one or more of us has been involved in since 1981. We first compare the freeze mortality during two storms (1981 and 1992) of butterflies clustering on tree boughs versus tree trunks. We then present field experiments that address two major and two derivative questions. First, during the overwintering season in Mexico, do tree trunks provide a 'hot water bottle effect', i.e. is the temperature of the trunk surface of oyamel trees sufficiently warmer than ambient to provide protection for the butterflies against freezing to death during clear cold nights and severe winter storms? If yes, is the freezing advantage greater on large than on small trees? Second, is the temperature of the trunk surface during the warmer parts of the day sufficiently lower than ambient such that the monarchs can conserve their stored lipids to a greater extent than monarchs clustering on the tree boughs? If yes, do larger trees provide greater cooling than smaller trees? And finally, since our results confirm that the thermal moderation was greater on larger trees, we compare a data set that we gathered in 1994 on oyamel diameters and ages in an overwintering colony with a published data set on ages and diameters in disturbed and undisturbed oyamel forests

Fig. 1. (a) Branch clusters of monarch butterflies on the boughs of an oyamel fir (*Abies religiosa*), located near the 'Carditos' area on the west face of Cerro Pelon in the Monarch Butterfly Biosphere Reserve, State of Mexico; 4 February 1978. Original Kodachrome by L.P. Brower. (b) A trunk cluster of monarch butterflies on an oyamel fir (*Abies religiosa*), located in the 'Ojo de Agua' ravine on the southwest face of Cerro Pelon in the Monarch Butterfly Biosphere Reserve, State of Mexico; 13 January 2006. Original digital image by L.P. Brower.

(Manzanilla, 1974). Putting all the pieces together leads us to conclude that management of the Monarch Butterfly Biosphere Reserve would be well served by a conservation policy that promotes old-growth forests.

Methods

Study sites

The data were collected in the Sierra Chincua and Cerro Pelon overwintering areas that are located on two separate mountain massifs in the Monarch Butterfly Biosphere Reserve in the states of Michoacan and Mexico. For detailed maps of these areas, see Slayback *et al.* (2007), and for colony locations dating back to 1977, see Calvert & Brower (1986), Missrie (2004), Slayback *et al.* (2007) and Slayback & Brower (2007).

The overwintering season

Monarchs generally begin arriving at their Mexican overwintering sites around 1 November, and usually all the survivors leave on their return migration to the Gulf Coast states by the end of March (Brower, 1995). We defined the overwintering season as extending from 30 October through 1 April to incorporate 22 full weeks. We also defined daytime as 8:00 to 20:00 hours and night as 20:00 to 8:00 hours so that the coldest hours, which usually occur shortly after sunrise, would be included in 'nighttime'.

Observations of storm mortality on oyamel trunk and bough clusters on Cerro Piedra Herrada

We obtained mortality data in the Cerro Piedra Herrada overwintering colony in the state of Mexico during the 1991–1992 overwintering season. The colony was located close to the position it had occupied during 1977–1978 (Calvert & Brower, 1986). Calvert revisited the area in mid-January, 1992, and observed that at least 20 large oyamels were festooned with monarchs in a colony that occupied 0.6 ha (Brower, field notes). Following his visit, one of the wettest late January–early February periods on record enveloped the overwintering region (Garcia, 1997). After 4 days of heavy intermittent rain (31 January–3 February 1992), a major northern cold front passed through, and on the morning of 4 February the skies were extremely clear throughout this part of Mexico (Brower, field notes). Radiational heat loss that accompanied the clearing sky was indicated by patches of ice and frozen soil along the trail leading up to the colony, and at noon the temperature inside the colony was 2.5 °C. Tens of thousands of monarchs littered the ground and understorey vegetation; only three oyamels still had clusters in what was now a remnant 0.1-ha colony, and Culotta (1992) subsequently reported extensive monarch butterfly mortality in the entire overwintering region.

Brower and colleagues collected monarchs in their clinging postures from the boughs of a young 4-m high oyamel fir and the trunk of an older oyamel about 50 cm in diameter. We put the butterflies individually into glassine envelopes, then into plastic bags, and transported them in a backpack by car to Mexico City where they were kept at cool ambient temperatures overnight. The next morning between 10:30–11:30 AM, they were warmed beneath a desk lamp and flight-tested by releasing them individually from the second story hotel window into full sunlight. As in Calvert *et al.* (1983), survival rates were determined by recording butterflies that were able to fly normally, or were flight impaired, moribund, or dead. We then compared this Herrada mortality with that observed in the Sierra Chincua colony following the January, 1981, winter storm (data from Calvert *et al.*, 1983).

Instrumentation

Weather station on Chincua. Because no official weather stations are located inside the Monarch Butterfly Biosphere Reserve (MBBR) (Garcia, 1997; Ramirez, 2001), we set up an electronic weather station (WeatherHawk, Model 232, Logan, UT, USA) on 24 November 2004. We located the weather station on the eastern side of the Sierra Chincua in a level open grassy area on the grounds of the El Llano las Papas Field Station (100°16′5″W, 19°39′42″N, elevation 3160 m). The area is surrounded by oyamel forest, is within the elevational range of the overwintering colonies on the Sierra Chincua and is only 2–4 km ESE of where monarch colonies have formed every year (Missrie, 2004; Brower, field notes) since the first Chincua colony was discovered in 1975 (Brower, 1995; Taylor, 1999). The WeatherHawk, located only 80 m from the beginning of our experimental transect, recorded temperature once each hour, providing average, minimum and maximum temperatures over the previous hour. While we would have preferred to place the weather station in an open area closer to sites traditionally occupied by overwintering monarchs, security reasons prevented this.

Thermal measurements on the oyamel trees. We used small (1.57 × 0.67 cm) digital temperature recording devices (Model DS1921G-F50 Thermochron iButtons, Maxim Integrated Products, Dallas Semiconductor, Embedded Data Systems LLC, Lawrenceburg, KY, USA). These measure temperature in 0.5 °C increments with a range of −40° to 85 °C and a stated accuracy of ± 1 °C. We programmed the thermochrons to record one instantaneous reading each hour on Chincua and one reading each 20 min on Pelon. We increased the frequency of recording on Pelon because the thermochrons were left in place for a shorter period of time (4 February to 3 March 2008 rather than all winter, with each able to record a maximum number of data points) and because doing so provided greater resolution in the temperature records.

We analysed all temperature records from Chincua as recorded directly in the field. All Pelon temperature records were adjusted after calibrating the thermochrons by obtaining comparative readings under identical laboratory conditions. They were spread out in a plastic bag, with each recording the temperature every 20 min for 12 h under refrigeration (35 readings averaging 3.1 °C) and for 14 h at room temperature (41 readings averaging 22.0 °C). We then compared the readings for each pair of thermochrons

Table 1. Diameters of trees on which temperatures were measured. The large category at each site included the largest trees available, while the small category included trees that stood alone and were smaller, yet still reached the canopy.

	Sierra Chincua		Cerro Pelon	
Size category	Mean + SD (*n*)	Range	Mean + SD (*n*)	Range
Large	69.3 + 14.7 cm (5)	57.3 to 91.8	74.3 + 2.8 cm (6)	68.7 to 76.4
Small	45.8 + 8.9 cm (5)	36.1 to 55.1	32.8 + 5.9 cm (6)	26.5 to 40.2

that were used in the field (two from the same tree, one on the trunk and one on the post, see below); when the average temperature of one was higher than the other, we subtracted that difference from the readings of the first so that each pair yielded identical averages. The temperature adjustments between pairs of thermochrons averaged $0.47 \pm 0.39°$ (mean $\pm$ S.D.) for nightly lows and $0.28 \pm 0.16°$ for daily highs. Because the ambient low temperatures were near the refrigeration temperature, we applied the calibration from refrigeration to all overnight low temperatures. Ambient high temperatures were near the average of the refrigeration and room temperature readings, so we applied the average of these calibrations to all daytime high temperatures.

Ambient forest temperatures were measured by mounting the thermochrons on 1.9 cm diameter dowel posts from which we had chiseled off a 3.2 cm long $\times$ 0.6 cm deep section from one end so as to have a flat surface facing the ground. We applied a 1.6 $\times$ 1.6 cm piece of female Velcro to the chiseled surface and further secured it with a small tack. Each post, coated with flat black paint, was mounted perpendicularly on the magnetic north side of the trunk. We applied male Velcro to the base of each thermochron to attach it to the female Velcro, a procedure that allowed us to remove the thermochrons for data downloading or replacement at approximately 80-day intervals. The lengths of the posts were cut so that the centre of each thermochron was mounted 6.4 cm (2.5 inches) distant from the trunk surface and approximately 2 m above the ground. We were careful in standardising these distances so that the thermochron measurements would consistently represent ambient temperatures beneath the forest canopy. Henceforth in this paper, the temperatures measured on the post are referred to as 'forest ambient'.

To measure the trunk surface temperatures, we cleaned lichens off the trunk and used two small tacks to fasten a 3-cm long strip of female Velcro vertically onto the trunk about 2 cm from the east side of the base of the post upon which we had attached the ambient thermochron. We then applied male Velcro to the base of the thermochron and attached it to the Velcro strip. Our analyses were based primarily on comparing the minimum overnight temperatures reached on the trunk and the post 6.4 cm away from the trunk, and on comparing the maximum daytime temperatures reached in these two locations. In addition, we compared the temperature regimes of the trunk, forest, and an open clearing as measured by the WeatherHawk, and we examined how the pattern of thermal buffering changed through the 24-h daily cycle. If the temperature readings on the posts were influenced by the trunk thermal regime, then the difference that we recorded between post and trunk would underestimate the actual difference between forest ambient and trunk temperatures. Thus, the differences reported here are conservative.

Thermochron deployment on the Sierra Chincua. On 30 November 2004, we set out post and trunk thermochrons on 13 oyamel firs in a largely closed but disturbed oyamel fir forest along a 700-m transect traversing a 3270-m high ridge behind Chincua Station. Even though monarch colonies have never been reported here, the general forest characteristics including tree density, size distribution, slope, elevation, and southern exposure are similar to the areas where monarchs do form colonies on the Sierra Chincua massif. We used a Garmin Vista global positioning system (GPS) unit and surveying techniques to locate our positions, and plotted them on an orthorectified Ikonos satellite image (1-m resolution) taken on 22 March 2004. The general area can be seen on Anon. (1999). The trees were not specifically chosen to represent large and small sizes, and for our analysis, we used the median tree size as the division between large and small categories (Table 1).

The thermochrons were kept in these locations from 30 November 2004 to 1 April 2005, from 30 October 2005 to 1 April 2006, and from 30 October 2006 to 1 April 2007. A few thermochrons and posts were vandalised or stolen, and some data became overwritten, so a few trees had to be deleted from analyses after 2005; thus, we had temperature records for 10 of the 13 trees and chose the most complete sets of temperature records for each of the comparisons we analysed.

The WeatherHawk station was 80 m SSW and 8 m lower in elevation than the first tree on the transect (ID number C81). C81 was located at 19°39′44″N, 100°16′5″W at an elevation of 3170 m. The next eight trees ran up the mountain in a NNW direction (25° west of N). The remaining four trees continued in a NNE direction (30° east of N) over the crest (about 3270 m) of the mountain down to about 3250 m. The 13 trees were at intervals of 44 to 76 m. while the highest trees (C-108 and C-111) were at 3270 m. The slopes at the 13 trees, measured with a clinometer, ranged from 0 to 23°.

During the 154 day overwintering period (30 October–1 April), from 2004 to 2007 we recorded a maximum of 29.0 °C and a minimum of −2.5 °C on the posts beneath the forest canopy along the 13-tree transect. During the same period, the WeatherHawk station recorded a maximum of 30.4 °C and a minimum of –8.6 °C.

Thermochron deployment on Cerro Pelon. On 4 February 2008, we placed thermochrons on six large and six small diameter oyamel trees in a dense mixed coniferous forest including oyamels, pines, and cedars growing on a 10°–20° slope on the south face of the western side of Cerro Pelon, a 3500-m elevation volcanic peak bordering the states of Michoacan and Mexico. The general area can be seen on Anon. (1974). We located and plotted our positions as described above. We intentionally chose six of the largest oyamels that were not close to another tree (Table 1). For comparison, we chose six of the smallest canopy trees that also were not close to another tree. The slope led down to a 9-ha flat open area, the Llano de los Tres Gobernadores. The elevation of the llano is 3020 m, and the uppermost trees that we used were at 3050 m. The trees occupied a rectangular area about 80 m E-W by 135 m N-S, and the geographical centre of the area was at 19°23′04″N by 100°15′22″W. The area occupied by the trees is at the same elevation and approximately 350 m east of where the Ojo de Agua monarch overwintering colony has been located in numerous years. Temperatures from 5 February through 2 March 2008 beneath the forest ranged from a maximum of 21.5 °C to a minimum of 1.0 °C along this transect. During the same period, the WeatherHawk at Chincua Station recorded a maximum of 26.2 °C and a minimum of −3.6 °C.

Ages and growth rates of oyamel firs in three forests

During the 1984–1985 overwintering season, we measured and, using an increment borer, cored oyamel trees that were festooned with monarchs inside an approximately 1.0 ha overwintering colony located at 19°40′8″N latitude 100°17′56″W longitude. The colony was at an elevation of 3048 m in the eastern branch of the Arroyo Hondo in the Sierra Chincua (Glendinning & Brower, 1990; Anon., 1999). Monarchs have overwintered in this arroyo for many of the past 30 years (Calvert & Brower, 1986; Missrie, 2004; Brower, field notes). We measured the diameters of 62 trees at breast height and obtained cores and counted annual rings from 60 of them. We then compared these age and size data with data from two oyamel forests reported in Manzanilla (1974). His first forest ('Rodales de Bosque Virgen') had no evidence of human intervention or forest fire, whereas his second ('Rodales de Bosque Natural') had been heavily affected by uncontrolled logging and forest fires. To avoid confusion, we renamed his second category 'disturbed forest'. Manzanilla (1974) did not give detailed locations other than to indicate that the forests were among several that he studied in the Transverse Neovolcanic Belt that extends across Mexico between latitude 19–20°N (Brower, 1995). All known major monarch colonies occur within this latitudinal range and within a longitudinal band from 99°40′ to 100°50′W (Slayback *et al*., 2007).

Lipid conservation

The cooler temperatures of the trunk compared to forest ambient during daytime suggest that monarchs may be able to conserve lipids because of a lowered metabolic rate, even though greater warmth at night may partially counterbalance the daytime gain. We assessed the overall lipid savings of monarchs resting on tree trunks compared to those remaining at ambient forest temperatures by combining three parameters: (i) an average wet weight of 538 mg for overwintering butterflies in Mexico (Alonso & Brower, unpubl. data); (ii) an overwintering period that lasts 154 days (30 October–1 April) (Brower *et al*., 2006); and (iii) the empirical relationship between body temperature and metabolic rate as measured for adult California monarchs by Chaplin & Wells (1982). Thus lipid consumption in mg per hour, where T is temperature, is given by:

$$\mathrm{LC/hr} = [10^\wedge(0.048 * \mathrm{T}\text{-}1.37)\ \mathrm{ml\ O_2\ consumed/g\text{-}hr}] * 0.538\mathrm{g\ butterfly} * 4.8\ \mathrm{cal/ml\ O_2} * [(4.18\mathrm{J/cal})/(0.037\mathrm{J/mg\ lipid})]$$

Using this formula and our hourly temperature records for 10 oyamel firs on Sierra Chincua through an entire 154-day overwintering period, we calculated the potential cumulative lipid savings of monarchs resting on tree trunks compared to those at ambient forest temperatures. Because lost thermochrons deprived us of continuous records for a full winter, this analysis combined temperature records from two winters: 30 October–19 December 2005 and 20 December 2004–1 April 2005.

Comparison of weather station and forest temperatures

To visually represent the thermal buffering provided by the forest cover and by the trunk microhabitat within the forest, we graphically compared the hourly temperature of one tree trunk (C-81), the ambient forest measured next to the same tree, and the weather station, during a 4-day clear period from 17–21 January 2008.

Statistical analyses

We used SPSS 16.0 (SPSS, 2008) and Zar (1996) to conduct all statistical analyses. Comparisons of trunk and forest ambient readings were made using a repeated measures general linear model for both daily high and nightly low temperatures. The effect of tree size was evaluated by calculating the difference between trunk and forest ambient temperatures and comparing these differences between large and small trees, using repeated measures and *t*-tests. The extent of thermal buffering during extremely cold nights and warm days was analysed by a univariate general linear model with the time series component of the data (readings over 27 days) incorporated through a tree effect, evaluated as both random and fixed (SPSS, 2008; Z. Dietz, pers. comm.).

Results

Storm mortality in the Cerro Piedra Herrada and Sierra Chincua colonies

The Herrada colony mortality results are summarised in Table 2 along with the mortality data for the 1981 storm in Chincua. Survival was significantly higher in both studies for monarchs clustering on the oyamel trunks compared to those clustering in the bough clusters. In the Chincua colony, 78% of the monarchs collected from the trunk clusters flew normally compared to 56% from the bough clusters. In the Herrada colony 43% of the monarchs collected from the trunk flew normally, whereas only 5% collected from the bough clusters did so. The rest were flight impaired, moribund, or dead.

Table 2. Comparative survivorship of monarch butterflies clustering on oyamel boughs vs. clustering on tree trunks following severe winter storms in the Sierra Chincua colony during January 1981 and in the Herrada colony during February 1992. The 1981 Chincua data are from Calvert *et al.* (1983).

	Collected from	
Location	Boughs	Trunks
Chincua, 24 January 1981*		
Alive	61	91
Moribund or dead	48	26
Total	109	117
Percentage alive	56%	78%
Herrada, 4 February 1992†		
Alive	1	9
Moribund or dead	19	12
Total	20	21
Percentage alive	5%	43%

*Chi-squared with Yates correction = 11.22, d.f. = 1, $P < 0.001$; †Chi-squared with Yates correction = 6.04, d.f. = 1, $P < 0.025$.

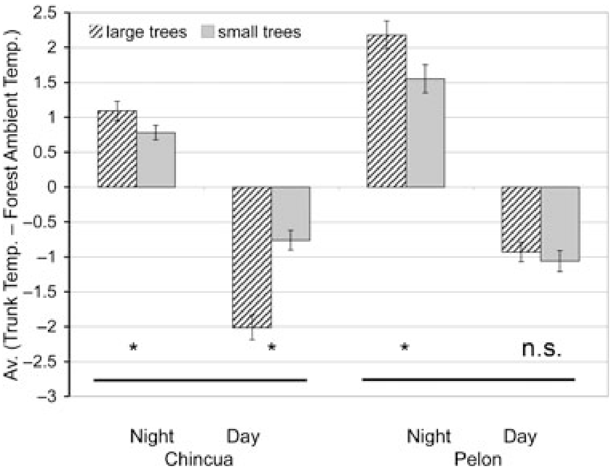

Fig. 2. Average differences (°C) between trunk and forest ambient temperatures for both the nightly minimum and daily maximum temperatures of large and small oyamel trees on the Sierra Chincua and on Cerro Pelon. Error bars mark the 95% C.I. of the means. *indicates significant, ns, not significant. In both forests, nightly trunk temperatures are significantly warmer than ambient, and daytime trunk temperatures are significantly cooler than ambient. Large trees produced a significantly greater difference during cold temperatures in both forests and during daytime maxima on the Sierra Chincua.

Microclimate of tree trunks

Differences between trunk and forest ambient temperatures. Oyamel fir trunks significantly moderated the microclimate (Fig. 2). From all 10 trees (five large and five small) over 28 days (1–28 February 2006) on Sierra Chincua, the minimum temperature of the trunks was higher at night than forest ambient by 0.9 °C (repeated measures $F = 17.14$, d.f. = 1,18, $P = 0.001$), while the daily maximum temperature of the trunks was 1.4 °C lower (repeated measures $F = 5.73$, d.f. = 1,18, $P = 0.028$). We found similar microclimatic buffering on Cerro Pelon (Fig. 2); again, the trunks gave significantly higher minimum readings at night than forest ambient for all 12 trees (5 February–2 March 2008), averaging a difference of 1.7 °C (repeated measures $F = 18.94$, d.f. = 1, 22, $P < 0.001$). The difference was reversed during the day, when daily maximum trunk temperatures were 1.1 °C lower (repeated measures $F = 9.82$, d.f. 1, 22, $P < 0.005$).

When all comparisons are combined from both sites (10 trees from Chincua and 12 from Pelon), the minimum surface temperatures of the firs at night averaged 1.4 °C warmer than ambient forest temperatures, and the maximum surface temperatures of the firs during the day averaged 1.2 °C cooler than ambient.

Effect of tree size. Tree size (Table 1) influenced the extent of microclimatic buffering (Fig. 2). At night, the larger trees from Sierra Chincua averaged small but significantly warmer temperatures above forest ambient than did small trees (1.1 °C higher than ambient compared to 0.8 °C; with measures as replicates, $t = 3.47$, d.f. = 278, $P = 0.001$). Furthermore, large trees remained significantly cooler during the day than did small trees (2.0 °C cooler than ambient compared to 0.8 °C; $t = 10.90$, d.f. = 278, $P < 0.001$). In the Pelon data set (Fig. 2), the large trees also gave greater thermal buffering during the night (2.2 °C compared to 1.5 °C; $t = 7.03$, d.f. = 295, $P < 0.001$), but there was no difference based on size during the day (0.9 °C compared to 1.0 °C; $t = 1.46$, d.f. = 295, NS).

Daily patterns. Based on our trunk and forest ambient records from the 12 trees on Cerro Pelon across 27 days in 2008, the tree trunks were regularly 1 °C warmer than forest ambient at night (20:00–8:00 hours), and slightly more than 1 °C cooler than forest ambient during the day (8:00–20:00 hours) (Fig. 3). The air warmed rapidly from 8:00 to 10:00 hours, and the greatest daytime differences occurred around 11:00 hours. The daytime differential then lessened through the afternoon as the trunk slowly warmed. Between 18:00 and 20:00 hours, the ambient temperature dropped rapidly, and, after 20:00 hours, the thermal mass of the tree trunks kept the temperature of the trunk surface constantly higher throughout the night.

Extent of microclimatic buffering in relation to cold and warm extremes. As shown in Fig. 4a, the difference between the nightly low readings is greater on relatively cold nights, reaching an average of + 2.7 °C when nightly low temperatures fell to 1 °C. In contrast, the difference between the daily high temperatures of the trunk and forest ambient readings (Fig. 4b) is greater on warmer days, reaching an average of –1.8 °C when daily high temperatures rose to 20 °C. Thus, the colder it gets at night and the warmer it gets during the day, the more the butterflies benefit by clustering on tree trunks. Both regressions are highly significant: for nightly lows, $y = -0.315x + 3.043$, $R^2 = 0.206$, $P < 0.001$; for daily high temperatures, $y = -0.126x + 0.751$, $R^2 = 0.102$, $P < 0.001$. The slope for low temperatures is also significantly greater than that for high temperatures ($t = 2.69$, d.f. = 320, $P < 0.01$; Zar, 1996). Thus, there is greater thermal moderation by the tree trunks against extreme low temperatures than against extreme highs.

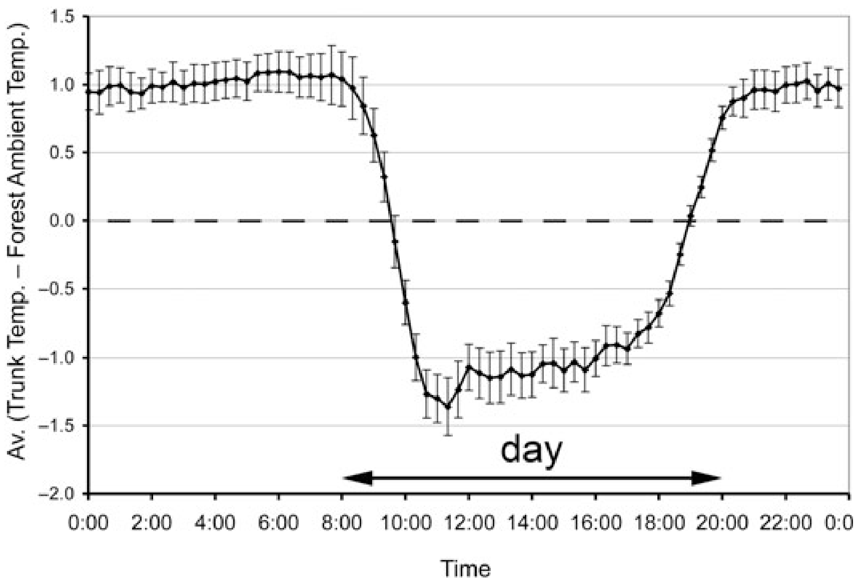

Fig. 3. Daily pattern of the difference between trunk and forest ambient temperatures (°C) recorded over 27 days on Cerro Pelon beneath the forest canopy. For each 20-min interval, the data represent the mean and 95% confidence intervals for 324 records (12 trees over 27 days). The trunk temperature averaged about 1 °C warmer at night and slightly more than 1 °C cooler during the warm part of the day. Note the rapid shifts from 08:00–10:00 and 18:00–20:00 hours.

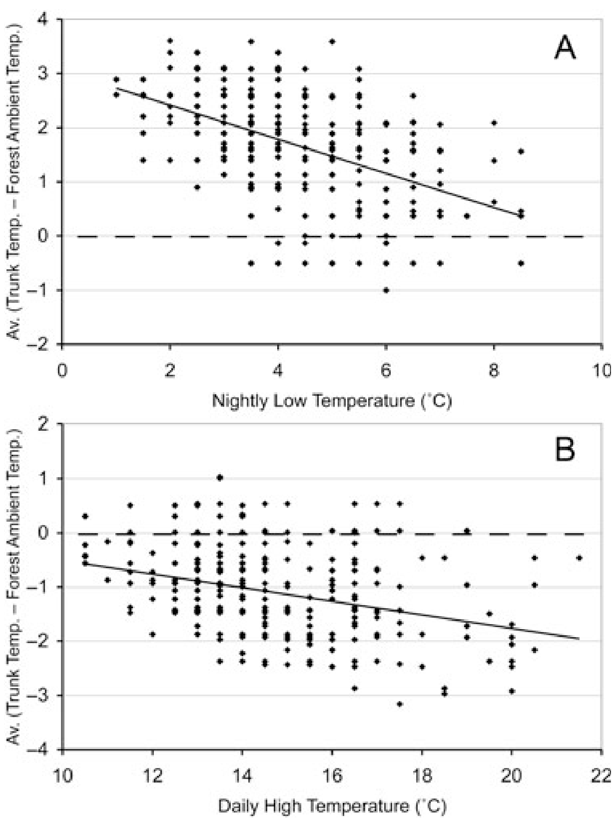

Fig. 4. Microclimatic buffering under extreme temperatures. The colder it gets during the night (a) and the warmer it gets during the day (b), the greater the buffering effect. Both regressions are highly significant (see text). The slopes also differ significantly, indicating that tree trunks provide greater thermal moderation against extreme low temperatures than they do against extreme highs.

Advantages of thermal buffering on tree trunks

Protection from freezing. The buffering of cold temperatures by tree trunks allows estimation of how much protection from freezing roosting monarchs would receive during extreme conditions. We extrapolated the regression of Fig. 4a, which shows that trees provide increased buffering during colder conditions, to estimate how much warmer the trunk would be at increasingly low sub-zero ambient temperatures. Then we used the survivorship of monarchs at these temperatures, as reported by Anderson & Brower (1996) and Larsen and Lee (1994), to predict survivorship rates at both forest ambient and estimated trunk temperatures. Our model predicts that during extreme cold spells, the temperature buffering provided by the tree trunks would substantially reduce the butterflies' risk of freezing whether the butterflies were wet or dry (Table 3).

Lipid Conservation. The analysis of cooler temperatures of the trunk compared to forest ambient during the daytime showed small though real savings of 3.7 ± 1.6 mg (mean ± 95% C.I.) of lipid over the full season. The net positive lipid value indicates that savings of lipid due to cooler daytime temperature exceeded the expenditure of lipids due to warmer nighttime temperature. Thus, monarchs resting on tree trunks for the entire overwintering period could save nearly 4 mg of lipid compared to monarchs resting in branch clusters or on other low mass substrates.

Microclimatic protection by the forest

Cumulative buffering by forest cover and tree trunks. Extreme cold and warm temperatures are substantially moderated within the forest by canopy cover (Calvert *et al*., 1982). This study demonstrates a further microclimatic moderation provided by the oyamel tree trunks. Figure 5 illustrates the cumulative effect by

Table 3. Predicted survivorship of wet and dry monarchs on tree trunks at various sub-zero ambient temperatures. Predicted trunk temperatures were estimated by extending the linear regression in Fig. 4a. Percentage of survival was estimated by applying the survival rates given in Anderson & Brower (1996)* to those temperatures.

		Percentage of survival at forest ambient temperature		Percentage of survival at predicted trunk temperature	
		Butterflies		Butterflies	
Forest ambient temperature (°C)	Predicted trunk temperature (°C)	Wet	Dry	Wet	Dry
0	+ 3.0	100	100	100	100
–2	+ 1.7	98	99	100	100
–4	+ 0.3	69	95	100	100
–6	–1.1	7	78	99	100
–8	–2.5	0	47	97	99
–10	–3.9	0	20	75	95

*Anderson & Brower (1996) determined that monarchs with water on their bodies begin freezing to death as the ambient temperature drops below –2 °C, with 100% dying below –7 °C. Those that are dry also begin freezing to death as the ambient temperature drops below –2°, with 100% dying below –15 °C.

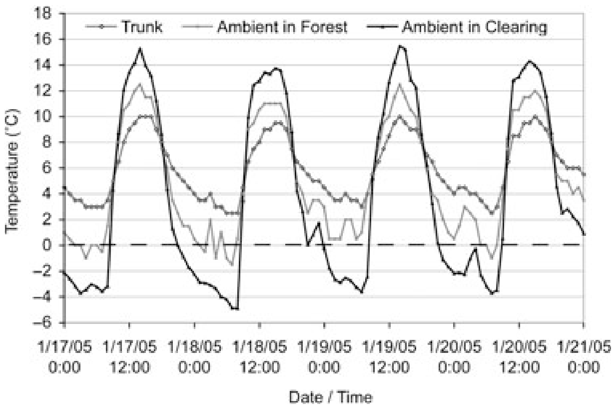

Fig. 5. Comparison of temperature records on the Sierra Chincua measured on four clear days in a clearing, inside an oyamel forest, and on the bark of a large oyamel inside the forest. Microclimate moderation is enhanced both by forest cover and by the heat capacity of the tree trunk. Note that the clearing has the greatest variation and the trunk the least.

plotting the measurements from 17–21 January 2005 for the single large oyamel fir (tree C81) located within the forest at the bottom of the Chincua transect, 80 m from the WeatherHawk weather station. The mean minimum temperature at the weather station was −4.0 °C, while that for the forest ambient records (the post attached to tree C81) was −0.8°, a difference of 3.2°. The mean maximum temperatures for the weather station and forest ambient at tree C81 were 14.7° and 12.0° respectively, showing the daily air temperature within the forest to be 2.7° cooler. The microclimate of the surface of the tree trunk is further moderated, being an average of 3.0° warmer than forest ambient at night and 1.8° cooler during the day. Thus, the cumulative effects of the forest canopy and the microclimate of the trunk result in monarchs clustering on this trunk being 4.5 °C cooler during the day and 6.2 °C warmer at night than they would be if they clustered in an open area.

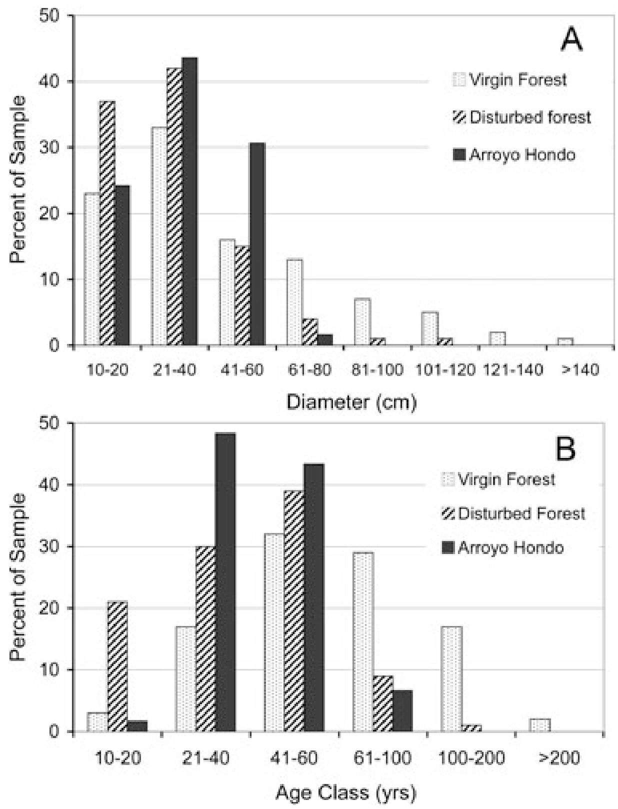

Fig 6. Distribution of oyamel (a) diameters and (b) ages in a virgin forest ($n = 331$, 328 trees), in a disturbed forest that was subjected to long-term harvesting ($n = 629$, 556 trees) (data from Manzanilla, 1977), and in a forest with monarch clusters on them in the Arroyo Hondo colony during the 1984–1985 overwintering season ($n = 60$ trees, data from this paper). Manzanilla's disturbed forest and the Arroyo Hondo forest indicate severe disturbance compared to the virgin forest. The largest reported oyamel diameter is 180 cm (see text).

Ages and growth rates of oyamel firs in three forests. Figure 6 presents a comparison of the ages and diameters of 60 oyamel trees on which monarchs were clustering during the 1984–1985 overwintering season in the Arroyo Hondo with Manzanilla's (1974) virgin and disturbed oyamel forests. The ages of the Arroyo Hondo oyamels ranged from 16 to 84 years (mean = 42.3; SD = 12.0 yrs), and their diameters (dbh) ranged from 8.1 to 65.3 cm (mean = 32.8 cm; SD =14.3). Calvert (2004) reported a similar average diameter (31 cm) for oyamels in the Arroyo Zapatero (Sierra Chincua) colony during the 1978–1979 overwintering season. The oyamels in Manzanilla's disturbed forest have similar size and age ranges to the Arroyo Hondo trees, whereas those in his virgin forest ranged higher in both age and size classes, with some trees older than 200 years.

Discussion

Thermal buffering of tree trunks

The evidence presented in this paper documents the microclimatic buffering that occurs on tree trunks. The answers to the questions we posed initially are that, yes, the trunks of oyamel firs in the overwintering grounds in Mexico are warm enough during cold nights to increase protection for the clustering monarchs against freezing and cool enough during the day to lead to conservation of their stored lipids. Furthermore, larger trees provide greater thermal advantages than smaller trees. The effect of tree size was significant during overnight minimum temperatures at both sites and significant during daily high temperatures on Chincua, while greater variance obscured the effect of tree size during the day on Pelon. Protection against freezing and lipid conservation are part of a suite of advantages provided by the oyamel forest microclimate that allow monarchs to overcome the challenging conditions of surviving the winter at high elevations in Mexico.

The temperature of the surface of a tree trunk is determined by the conduction of heat to and from the inner wood layers of the tree and by conduction and convection with the surrounding air (Geiger *et al*., 2003). Tree trunks warm and cool more slowly than air because of the high specific heat (the energy required to raise one g one °K) of wood. Measurements of the specific heat of wood samples have ranged from 1.2 J/g°K (oven-dried wood at 7 °C) to 1.9 J/g°K (wood with 20% moisture content at 27 °C) (Simpson & TenWolde, 1999). In contrast, dry air is a thousand times less dense than wood, with a specific heat of only 0.0012 J/cm^3 °K (CRC, 1992). Living trees with water in their tissues would have an even higher specific heat than the wood samples described above since the specific heat of water is higher, at 4.2 J/g°K (CRC, 1992). The moisture content of living trees is shown in measurements of five species of fir (balsam, grand, noble, Pacific silver, and white), which ranged from 25–49% for heartwood and 53–63% for sapwood (Simpson & TenWolde, 1999). The moisture content of oyamel fir trunks in the overwintering area may be even higher; water gushed out from 12 of the 60 trees when we cored them. The slow rate of warming and cooling of oyamel trunks compared to that of air is responsible for the modified microclimate that resting monarchs encounter.

Once on the tree trunks, the butterflies will adjust to the more stable temperature regime they encounter. The masses of tree trunks are so much greater than that of the butterflies clustering on them that the exchange of heat is effectively unidirectional. At night, heat stored in the tree trunk is transferred outward through the butterflies' bodies, while during the day, the direction of heat exchange is reversed. Both the pockets of air trapped in the tightly clustering butterflies' wings and the insulative capacity of the bark may contribute to maintaining separate thermal environments on the tree trunk. As a result, the thermal environment for butterflies resting on trunks is different from ambient conditions only a few cm away.

Advantages of clustering on tree trunks versus tree boughs

In contrast to the temperature buffering provided by the tree trunks, bough clustering provides butterflies with a very small thermal benefit (Brower *et al.* 2008b). Branch cluster temperatures are very close to ambient forest temperature, almost certainly because wind currents rapidly change conifer needle temperatures (Tibbals *et al*., 1964; Chown & Nicolson, 2004). The greater thermal buffering of the trunks that we found is sufficient to account for the higher observed survivorship during harsh weather of monarchs on trunks compared to those in boughs (Table 2).

Freeze avoidance. Anderson & Brower (1996) experimentally determined that the risk of freezing to death for a wet monarch butterfly increases precipitously as temperatures drop from −4 to −6 °C. Their findings were verified on 14 January 2002 in the Sierra Chincua and Sierra Campanario colonies when a severe winter storm wetted the butterflies and the temperature was estimated to have dropped to between −4° and −5 °C. About 75% of the butterflies were killed, and close to 500 million perished in the overwintering region (Brower *et al*., 2004). Fink *et al.* (unpubl. data) have demonstrated that the risk of freezing is also affected by the duration of the cold temperature that a wetted butterfly experiences. When wet butterflies were held at −4 °C, median survival time was greater than 20 h, and more than 75% of butterflies survived for 10 h. In sharp contrast, at −5 °C the median survival time dropped to 32 min, and at −6 °C, to 5 min.

Three different processes result in increased freeze protection for butterflies clustering on tree trunks beneath the forest canopy vs. clustering on the tree boughs (Fig. 1a,b): because of the hot water bottle effect, (i) the minimum temperature on the trunk is not as low as the minimum forest ambient temperature at night (Fig. 5); (ii) the duration during which the trunk temperature remains below freezing on a cold night is likely to be shortened; and (iii) the microclimatic buffering by the trunk is greater during colder temperatures (Fig. 4a). Furthermore, as we found in both the Chincua and Pelon oyamel forests, increased microclimatic buffering is produced by larger trees (Fig. 2), thereby confirming the importance of large diameter trees for the increased quality of an overwintering site. The same thermal relationships of trunk and forest ambient temperatures and tree size were found by Lear (1998) in her pilot study on pines in Virginia.

Records from 1961–1987 at the Rio Frio Ixtapalucan weather station (elevation 2980 m) southeast of the Monarch Butterfly

Biosphere Reserve summarised in Ramirez (2001) indicated an absolute minimum temperature in February of −9 °C. Madrigal-Sánchez (1967) independently reported a low of −11 °C in an oyamel forest in the Valley of Mexico, and according to Manzanilla (1974), the lowest temperature on record near the high-elevation oyamel forests in the Transverse Neovolcanic Belt also was −11 °C. The lowest recorded temperature at our Chincua weather station from November 2004 through March 2008, a period when there were no severe winter storms, occurred on 8 January 2006 and was −8.6 °C. Based on Anderson & Brower's (1996) findings and the extrapolation of trunk temperature given in this paper, if a combination of butterfly wetting with an ambient forest temperature as low as –10 °C were to occur, it is likely that branch cluster survival would decline to a catastrophic 0%, while up to 75% would survive on the tree trunks (Table 3).

Lipid conservation. By reducing the highest temperatures reached by butterflies during the warmest times of day, microclimatic buffering by tree trunk surfaces will lower the butterflies' metabolic rates. Our analysis shows that the lipid savings achieved by remaining cooler during the day more than offsets the lipid costs of remaining warmer during the coldest times of night. This overall difference appears real, although small. The cooling effect is even greater during very warm conditions (Fig. 4b). If this leads to longer periods of quiescence, and therefore less time in metabolically demanding flight (Masters *et al*., 1988), the lipid savings could be substantially higher than we have calculated.

Walford (1980), Brower (1985), and Brower *et al*. (2006) determined that fall migrants increase their mean lipid contents as they migrate through Texas and northern Mexico by as much as 500%. Monarchs collected from bough clusters in a Sierra Chincua colony in December 1993 contained an average of 113 mg lipid, and fewer than 5% contained less than 30 mg (Alonso-Mejia *et al*., 1997). By March, the overwintering monarchs had metabolised about half of their lipid reserves, with nearly a third of the butterflies containing less than 30 mg. Hoth (1995) maintained that overwintering monarchs can replenish their lipids through nectaring on the understorey flora in the vicinity of the colony, but subsequent analyses (Alonso-Mejia *et al*., 1997) determined that up to 70% of the butterflies attempting to obtain nectar from flowers contained ≤ 15 mg lipid and were very likely on the verge of starvation (Brower, 1995). Both Brower (1995) and Alonso-Mejia *et al*. (1997) concluded that monarchs are unable to replenish their lipid reserves at the overwintering sites. It is therefore likely that the potential 4 mg lipid savings due to clustering on the cooler daytime trunk surface is important for the end-of-season butterflies with low lipid titers, increasing the probability that they will be able to make a successful spring re-migration.

Conservation implications of the current oyamel forest degradation

Our findings that freezing and energetic advantages increase with tree diameter suggest that if monarchs historically formed colonies in old-growth forests, they could have enjoyed substantially greater thermal buffering than they do now. The largest oyamel on record had a diameter of 180 cm (Anon, 2008), which is nearly three times that of our largest Arroyo Hondo oyamel (65 cm dbh). Considering the available area upon which to cluster, on a 10-m length of tree trunk, the 180 cm diameter oyamel would have about 56 m^2 of surface area compared to about 10 m^2 for our average-sized oyamel in the Arroyo Hondo (32.8 cm dbh). Therefore, in historical forests, because of the larger surface area of the bigger trees, more monarchs may have rested on tree trunks than on the boughs as they do in the current degraded oyamel forests (Calvert, 2004). If this were the case, then a higher proportion of butterflies would have survived severe freezing events in the historical forests.

The annual increase in diameter of our 60 Arroyo Hondo oyamel trees ranged from 0.30 to 1.46 cm/year (mean = 0.75 cm/year). It would therefore take from 120 to 600 years (average 240 years) to attain a diameter of a 180 cm, and likely longer because of the decelerating growth rate of larger trees. The diameter and age data for our oyamels in comparison with Manzanilla's (1974) virgin forest trees (Fig. 6) add to the evidence that all known overwintering colonies in Mexico occur in disturbed forests (Snook, 1993; Madrigal-Sánchez, 1994; Slayback & Brower, 2007; Brower, field notes). Anti-logging enforcement in the Monarch Butterfly Biosphere Reserve in Mexico is currently focusing on large-scale illegal operations (Ramírez *et al*., 2003; WWF-Mexico, 2004, 2008; Brower & Magruder, 2006; Barajas, 2007; Brower *et al*., 2008a) and has ignored the slower degradation caused by low intensity but sustained tree harvesting by individuals and by horse logging groups.

It is clear from our data and general thermodynamic considerations that the Monarch Butterfly Biosphere Reserve microclimate would benefit greatly by more careful monitoring and more effective enforcement against all modes of logging. Implementing these conservation activities would allow the forests to undergo long-term growth and produce larger trees, and, therefore, lower freezing mortality and help maintain the butterflies' lipid reserves.

Questions emerging from this study

Further studies are needed to expand our understanding of the relationship between monarch butterflies' survival and the microclimates they experience in the high-elevation oyamel fir forests in which they overwinter. Empirical data are needed to confirm that the buffering of trunk temperatures reported in this paper are translated into measurable buffering of butterfly temperatures. Because no severe cold event occurred in the 4 years in which we have collected extensive temperature measurements, we have not yet been able to measure the protection against butterfly freezing provided by the hot water bottle effect. Likewise, we do not know how different degrees of forest thinning affect the day and night temperatures of bough and trunk clusters. Also, we do not know how much the butterflies themselves contribute insulation that may further moderate both the cooler daytime and warmer nighttime temperatures.

The behavioural bases of cluster formation should be investigated further. For example, do monarchs show a preference for clustering on tree trunks, and do they compete for position as they form their clusters? Does such a preference, if it exists, change during the course of the overwintering season? Do butterflies prefer larger trees? Preliminary data indicate that there is a vertical temperature profile in the forest; how might this affect where the monarchs form and maintain their clusters? Finally, additional microclimatic advantages may accrue through clustering on larger diameter tree trunks (e.g., greater protection from wind and horizontally driven rain), and these should be explored.

These considerations bear significantly on the conservation management policy that, based on the findings in this study, should advocate old-growth oyamel forest succession.

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