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Freeze-protection of overwintering monarch butterflies in Mexico: critical role of the forest as a blanket and an umbrella

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Abstract. 1. At their high-altitude overwintering sites in Mexico, monarch butterflies frequently are subjected to sub-zero°C temperatures during December–March. Although monarchs have moderate supercooling ability, two ecological factors strongly influence their capacity to resist freezing: wetting and exposure to the clear night sky.

2. As shown in Fig. 2, 50% of a population of butterflies with water on their body surfaces freeze at warmer sub-zero temperatures (-4.2°C) compared to butterflies with no water on their bodies (-7.7°C). 100% mortality occurs, respectively, at -7.7°C and -15°C .

3. Comparative measurements of rainfall within a large overwintering colony in Mexico indicated that the intact canopy acts as an umbrella that reduces butterfly wetting during winter storms.

4. Variable experimental exposure of butterflies to the clear night sky indicated that openings in the forest canopy increases radiational cooling and causes monarch body temperatures to drop as much as 4°C below ambient air temperature. Monarchs under dense cover had body temperatures approximately the same as the ambient air temperature, but more exposed individuals had body temperatures below ambient in direct proportion to the degree of exposure. Consequently, forest thinning increases the probability that the butterflies will freeze to death.

5. Whereas both wetting and exposure are increased by disturbance of the forest canopy, the interaction of these two factors exacerbates freezing mortality during winter storms: 50% of dry and unexposed butterflies froze at -8°C , whereas wetted and fully exposed butterflies froze at only -0.5°C .

6. Butterflies inside and on the bottom of the fir bough clusters are better protected from wetting than those on the outside. This supports the hypothesis that the structure of the butterfly clusters has evolved through individual selection to avoid wetting.

7. The data strongly reinforce previous evidence that forest thinning should be totally prevented within and adjacent to the overwintering sites in order to minimize both wetting and exposure of the butterflies that synergistically increase winter mortality at the overwintering sites in Mexico.

Key words. Cryobiology, overwintering survival of *Danaus plexippus*, monarch butterfly, high altitude microclimate, *Abies religiosa* fir forest ecosystem, forest thinning, deforestation, endangered biological phenomenon, Sierra Chincua, Transverse Neovolcanic Belt, Mexico, experimental study, synergistic interaction of exposure and wetting, evolutionary advantage of clusters.

Introduction

The migratory cycle. Monarch butterflies, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), migrate each spring from remote winter refuges in the mountains of central Mexico to colonize a huge area of the eastern U.S.A. and southern Canada (Brower, 1995). After three to five generations of population expansion on abundant summer milkweeds, the autumnal generation responds to climatic cues of the approaching autumn equinox (cooler temperatures, shortening day lengths) by migrating southwestward to the same overwintering sites (Calvert & Brower, 1986; Herman 1985; Urquhart & Urquhart, 1977). There, clustering on the trees in dense colonies, most of the monarchs manage to survive 4–5 months, until cues of the impending spring equinox elicit mating behaviour and the northward remigration (Barker & Herman, 1976; Brower, 1985; Van Hook, 1993).

The high altitude overwintering sites of the monarch butterfly in tropical Mexico are characterized by a complex set of climatic and physiographic features (Brower, 1985; Calvert & Brower, 1986). The sites are found in forests of the Oyamel fir (*Abies religiosa* H.B.K., Pinaceae) between altitudes of 2900 and 3400 m, and are limited to five isolated mountain ranges within an 800 km² area in the Transverse Neovolcanic Belt of Mexico (Calvert & Brower, 1986). Minimum daily January–February temperatures in areas exposed to the sky at the Sierra Chincua sites ranged from +4.5 to –5.2°C during 1979, 1981 and 1984 (Calvert & Brower, 1986; Calvert *et al.*, 1983, 1986). The cool climate favours conservation of the butterflies' lipid reserves (Masters *et al.*, 1988), and helps maintain their physiological state of diapause which holds the reproductive cycle suspended (Herman, 1985); there is also sufficient sunshine to allow flight for water or nectar, or to avoid predators.

Cold-hardiness. During the coldest months (January and February), freezing temperatures are frequent at night, especially close to the ground in open areas (Calvert & Cohen, 1983; Calvert *et al.*, 1982, 1983; Alonso-Mejia *et al.*, 1993). Northern winter storms occasionally penetrate the Tropic of Cancer and cause snow, hail and freezing rain (Urquhart & Urquhart, 1976; Calvert *et al.*, 1983, 1989; Brower, 1985). Records from weather stations in the Transverse Neovolcanic Belt near the overwintering sites show that extreme minimum temperatures over the past 10–26 years have fallen as low as –9°C at several locations (Anon., 1982; review in Anderson & Brower, 1993). Although these stations are in exposed areas where conditions are more extreme than beneath the forest (Calvert *et al.*, 1982), many episodes of subzero temperature have been recorded in the butterfly colonies. For example, when clearing occurred after a prolonged winter storm in the Sierra Chincua during January 1981, the minimum temperature reached –4.1°C beneath the forest and –5.0°C in an adjacent open area of the forest. About 2.5 million butterflies were killed by this event (Calvert *et al.*, 1983). A similar weather pattern occurred in February 1992 which was estimated to have killed 83% of the butterflies in one colony that we visited immediately after the storm (Brower, in Culotta, 1992).

Using a standard cryobiological assay (Salt, 1961; Sømme, 1982), Anderson & Brower (1993) determined that adult

monarch butterflies collected at their overwintering site in Mexico are capable of supercooling and thus able to survive at body temperatures ranging from –1.5 to –14°C. The data are in agreement with Urquhart's (1960) earlier study in which he subjected groups of monarchs to 12 h of sub-zero temperatures and found complete recovery in some exposed to temperatures as low as –6.8°C. Below the minimum tolerable temperatures, the haemolymph freezes and either kills or permanently maims the butterflies. Anderson & Brower's (1993) study also determined that monarchs from various populations have a seasonal change in their mean supercooling points (Fig. 1).

The results of these cryobiological experiments, together with several microclimate studies at the Mexican overwintering habitats (Calvert & Brower, 1981; Calvert *et al.*, 1982, 1983, 1986; Calvert & Cohen, 1983; Alonso-Mejia *et al.*, 1992, 1993), suggested that the degree of cover provided by the forest canopy protects the monarchs from freezing by both blanket and umbrella effects. We therefore designed experiments to quantify the degree of wetting and exposure and their interaction on the cold-hardiness of monarch butterflies at an overwintering site located in the Sierra Chincua of the Transverse Neovolcanic Belt, in Michoacan, Mexico.

Wetting. Surface water on an insect tends to freeze at relatively high temperatures, and ice crystals can then radiate into the body by inoculative freezing, unless blocked by integument or inhibited by some internal factor (Block, 1982; Duman *et al.*, 1982; Sømme & Conradi-Larsen, 1979). The lowest temperatures at the Mexican overwintering sites usually occur during the night at the end of rain and snow storms when northern cold fronts push southwards resulting in clear skies. Even with no previous precipitation, exposed surfaces on clear nights become wet with dew (Calvert *et al.*, 1986; Alonso-Mejia *et al.*, 1992).

Exposure. As reviewed in Geiger (1980), incoming solar radiation is absorbed by objects depending on their albedo (reflectivity) and is then emitted as infrared radiation. At night, infrared emission continues without the sun's input. If an object is beneath cloud cover, tree canopy, etc., much of the energy is reflected and re-radiated back from the overhead cover and vegetation, and is regained by the object. However, if the object is directly exposed to the clear night sky, much of its contained energy radiates directly to the cold of outer space. This radiant cooling can cause the temperature of the object to fall and come into a thermal equilibrium below the ambient air temperature surrounding the object. The degree to which the object drops below ambient temperature depends on topography, altitude, the degree of exposure, the ambient air temperature, and the temperature of the substrate upon which the object is resting, such as foliage, the ground, etc. If the relative humidity is high, condensation on the colder surfaces results in dew formation.

This study. Our experiments were conducted in 1985 and 1986 at the major Sierra Chincua overwintering site in Mexico. We (1) analysed the effects of wetting on the supercooling points of the monarchs, and (2) determined the role of the forest canopy in protecting the butterflies from natural wetting. We also assessed how butterfly freezing is affected by (3) the degree of exposure to the clear night sky, and (4) by the interaction of exposure and wetting.

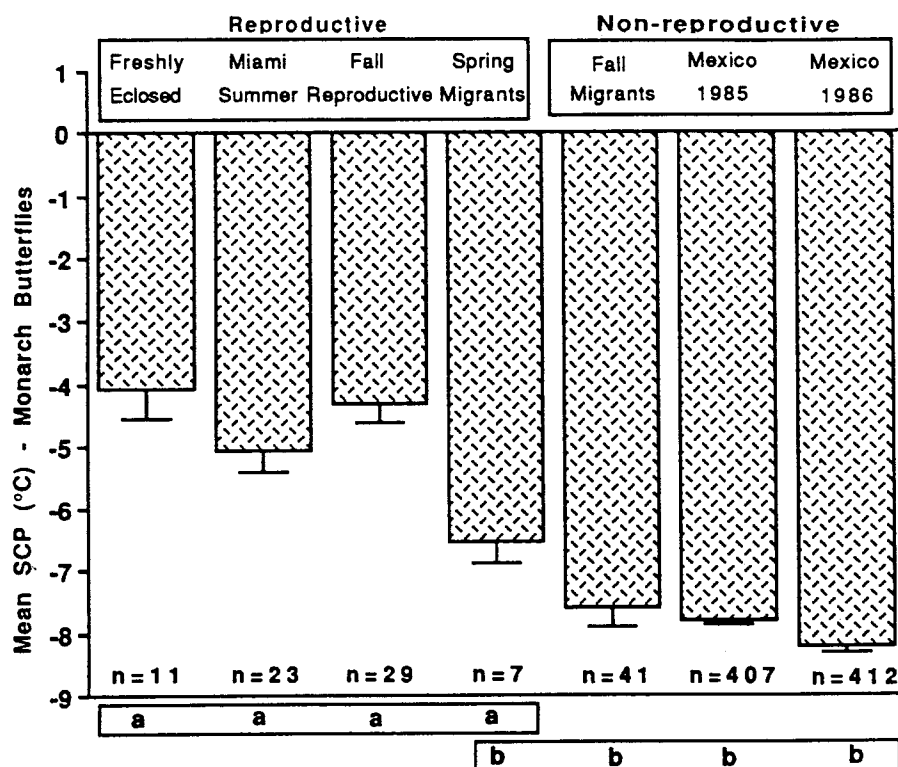


Fig. 1. Seasonal changes in experimentally measured supercooling points of dry monarch butterflies collected during various parts of their annual migration cycle. Samples designated 'a' are significantly different from samples designated 'b' (one-way ANOVA, Scheffe F test). The bars are standard errors of the means (modified from Anderson & Brower, 1993).

Materials and Methods

Experimental measurement of freezing point. We established a field laboratory during January–March of 1985 and January–February 1986 at a large monarch overwintering site on the Sierra Chincua near Angangueo, Michoacan, Mexico, as described in Calvert & Brower (1985). We measured the degree to which monarchs can supercool and thus survive subzero temperatures. Supercooling points – the body temperature at which freezing occurs – were determined for individual monarchs using a Haake A80 cooling bath powered by a Honda 3000 W generator. Six stoppered vials, each containing a live monarch, were immersed in the bath solution of ethylene glycol, and cooled at a rate of 0.44°C/min, the fastest rate possible with this cooling bath. A capillary tube through each stopper permitted air pressure to equilibrate. A small surface thermistor (YSI series 400) was taped to the anterior ventral abdominal surface of each monarch, for continual monitoring of body temperature. This non-invasive measurement was identical to the temperature within the thorax, as checked several times initially with a needle thermistor (YSI series 500, 34 gauge hypodermic probe). The thermistors were connected to a YSI telethermometer through a switch box, so that, during the controlled, cooling period, each monarch body temperature could be recorded at 1 min intervals. When the supercooling point was reached, it was detectable as a sharp elevation in body temperature – a result of the latent heat of fusion released as water freezes (Salt, 1961; Sømme, 1964). This

standard rate of cooling was necessary for comparative purposes, but different rates of cooling might result in different supercooling points (Baust & Rojas, 1985; Larsen & Lee, 1994). As previously determined by Anderson & Brower (1993) and confirmed by Larsen & Lee (1994), once frozen, monarchs are either killed outright or maimed and do not recover normalcy when moved to sunlight at ambient temperatures characteristic of clear days at the overwintering sites.

Wetting-effects of rain on the supercooling points. The first rain we observed fell on 27 February 1985, and lasted for 75 min, ending about 18.30 hours. The wind blew lightly up-slope from the northeast, as was the case in the three rainfalls which followed in March (see below). Immediately following the first rain, fourteen samples of butterflies were collected from positions of different exposure within the monarch colony (Table 1). The samples included butterflies from the surfaces of and from within both large and small bough clusters, from trunk surface clusters on the northeast and southwest sides of trees, and from ground vegetation in a small clearing.

Each sample consisted of ten butterflies that were individually plucked with large forceps and put together in one labelled zip-lock plastic bag for transport to camp. Each group of ten wet butterflies and its respective bag were weighed with an electronic balance within 39 min after collection. While still wet, five monarchs from each bag were individually tested for their supercooling points. They were then put into a nylon mesh cage to dry for 24 h, after which each was weighed individually. The

remaining five wet butterflies were allowed to dry in a separate cage for 24 h, tested individually in the same manner for supercooling points, and then weighed individually. The total amount of water (mg) on each group of ten butterflies and on the inner surface of their respective plastic bags was determined by subtracting the combined dry weights of the two butterfly subsamples plus the dry weight of the plastic bag from the total wet bag + wet butterfly weights. This difference – the total weight of water on the ten butterflies – was then divided by 10 to give the average weight of water (mg) per butterfly, as recorded in the column B of Table 1.

The experimental design thus allowed determination of the relationship between freeze-vulnerability, butterfly position within the colony, and the degree of wetting.

Wetting-effects of controlled spraying. We also ran supercooling point tests on butterflies that we sprayed with a fine mist of water from a hand-operated garden sprayer. Freshly collected monarchs were individually weighed, sprayed with from one to nine sprays, weighed again individually, and then each was tested for its supercooling point. In this experiment the supercooling point of the individual butterflies was related to the amount of surface water (mg) on their wings and bodies.

The overhead canopy – protection from wetting? A grid of 10 × 10 m quadrats was established within the monarch colony early in January 1985. To investigate the relationship between tree cover and wetting by rain, rainfall was measured at forty-three different points within the colony during each of three rain storms that occurred during March. We subjectively ranked the degree of canopy cover above each grid intersection on a scale of 1–5, with 1 designating completely exposed to the sky, and 5 designating the densest canopy cover. One styrofoam cup, 8 cm in diameter, was stapled to the top of a stake at 1 m height in the center of each of the twenty-one quadrats ranking 1 and 2 (the most exposed points); an additional twenty-two cups were set in the centre of the quadrats ranking 4 and 5 (the least exposed points). The volume of water in each of the forty-three cups was measured after each of the three storms using a calibrated plastic syringe fitted with a rubber tube. The total ml of rain per cup resulting from the three storms was then related to the degree of canopy exposure.

Exposure – the effect of radiational cooling. Another experiment was run on virtually cloudless nights during February and March 1985 to examine the relationship between monarch body temperature and degree of exposure to the clear night sky. In a large forest clearing near our campsite, four squares of plastic glasshouse shade netting were stretched horizontally about 1 m above the ground. Each was a square metre of woven mesh screen with a different shade quality. The degree of shading produced was quantified during direct sunlight at midday using a Licor pyranometer sensor with an LI-integrator. The quantity of sunlight reaching the sensor through each screen was divided by the average value of full sunlight measured immediately before and after the screen measurement. These values were 5%, 26%, 51% and 72% of the full exposure afforded through each screen, respectively. Plain wooden planks 20 cm wide were positioned about 50 cm below these screens and approximately 50 cm from the ground. On eleven different nights, live monarchs (immobilized by the nightly cold) that we collected from the colony clusters were placed on the planks beneath these artificial covers

(as well as under no cover, i.e. 100% exposure) with a thermistor taped to their abdomens (as in the supercooling point tests). At 04.30 hours we recorded both individual monarch body temperatures and ambient air temperatures beneath the netting next to each butterfly.

Results

Rain, position within colony, and supercooling points

Results of the experiments on the naturally wetted butterflies are in Table 1. Column A shows fourteen different locations from which the butterfly samples were taken. Column B gives the single mean measure of wetness of the ten butterflies collected from each position. The sample variances of the mg of water on the butterflies were estimated from the two mean values in each of six fir cluster contrasts (NE v SW, NE v Bottom, NE v Inside, SW v Bottom, SW v Inside, and Bottom v Inside) using Stateview 4.0 (Haycock *et al.*, 1992) and did not differ significantly: all *P* values were >0.14. One-way ANOVA followed by Fisher's posthoc Protected Least Significant Difference Test (SuperANOVA 1.11; Gagnon *et al.*, 1989) was used to compare the mean amounts of water on four of the branch cluster groups (NE, SW, Bottom, Inside): d.f. = 3 for position, 4, residual; *F* = 15.57, *P* < 0.012 for the effect of position; five of the six contrasts were significant (*P* < 0.05), but one, the bottom versus the insides of the clusters was not (*P* > 0.91). The data in column B therefore indicate that: (1) the butterflies on the side of the branch clusters exposed to the direction (NE) from which the rain storm approached were wetter than those on the leeward side; and (2) the butterflies on the bottom and inside of the branch clusters were better protected with 60% of the amount of water on them compared to butterflies on the exposed cluster surfaces. Further inspection of the data in column B suggests that the butterflies clustering on the tree branches and trunks were substantially drier than the butterflies clinging to the open vegetation growing amongst the trees.

Two overall pictures emerge from these data: (1) butterflies exposed to the direction from which the storm approached are wetter; and (2) within the clusters, the butterflies shield each other from the rain. The latter point is the first evidence adduced to date that the butterflies inside the clusters can remain drier during storms and has strong implications for the evolution of the clustering behaviour.

The data in column C indicate that the butterflies in the wetter samples froze at significantly higher temperatures than the butterflies collected in the drier samples, i.e. from the bottom or inside of the branch clusters (column C, comparison of the mean supercooling points in rows 1, 2, 5, 6, 7, 10 and 14 with rows 3, 4, 8, 9 and 11). A two-way ANOVA of the supercooling points of the forty wet butterflies in rows 1–4 and 5, 6, 8 and 9 indicated that the supercooling points were not affected by cluster size (*F* = 0.25, 1 d.f., *P* > 0.25), but were strongly influenced by the butterflies' position in the cluster and with respect to the direction of the rain (*F* = 16.1, 3 d.f., *P* < 0.0001); the interaction was not significant (*P* > 0.58).

The same two-way ANOVA was run on the supercooling points of the respective forty dry butterflies in column D and indicated that, once dry, the butterflies' supercooling points did

Table 1. Comparisons of the mean mg of water on monarch butterflies naturally wetted by rain and their mean supercooling points (\pm SE) while wet and after drying. The butterflies were collected from natural clusters within the Sierra Chincua overwintering colony after a rain storm which blew in from the north-east on 27 February 1985. The values in column B for the fourteen categories are averages of monarchs which were weighed together in groups of ten. The values in columns C and D are based on five individual monarchs drawn from the groups of 10 in column B (i.e. five were individuals tested while still wet, and five were individuals tested after drying). Column E gives the within-row significance (one-tailed *t* tests) between columns C and D. Both the exposure and position of the butterflies in the clusters strongly influence their wetting, and therefore their probability of freezing. See text for further statistical analyses.

A Sample location	B Mean surface H ₂ O (mg) per monarch	C Mean SCP while wet	D Mean SCP after dried	E <i>P</i> values. for C v D
Fir branch large cluster				
1. Northeast	64.5	-3.5 \pm 0.3	-7.8 \pm 0.6	<0.001
2. Southwest	32.2	-3.5 \pm 0.8	-8.4 \pm 0.6	<0.001
3. Bottom	18.7	-8.6 \pm 0.9	-6.5 \pm 0.8	=0.053
4. Inside	19.0	-8.4 \pm 0.8	-7.6 \pm 1.1	=0.29
Fir branch small cluster				
5. Northeast	72.4	-3.4 \pm 0.5	-6.6 \pm 0.8	=0.005
6. Southwest	55.4	-3.3 \pm 0.3	-8.1 \pm 0.6	<0.001
7. Top	27.7	-3.0 \pm 0.6	-6.2 \pm 0.6	<0.005
8. Bottom	14.7	-6.3 \pm 1.8	-9.1 \pm 0.9	=0.10
9. Inside	16.4	-7.9 \pm 1.0	-8.0 \pm 1.8	=0.48
Fir trunk				
10. Northeast	57.5	-3.2 \pm 0.2	-7.7 \pm 1.1	<0.005
11. Southwest	18.0	-5.6 \pm 1.3	-8.7 \pm 0.5	=0.032
Pine trunk				
12. Northeast	22.0	-7.7 \pm 0.5	-8.4 \pm 1.2	=0.316
13. Southwest	26.2	-8.6 \pm 0.7	-6.9 \pm 0.5	=0.045
Open vegetation				
14.	84.5	-3.1 \pm 0.2	-7.8 \pm 1.0	<0.001

not differ from each other either with respect to cluster size ($F = 0.326$, 1 d.f., $P > 0.57$) or position ($F = 0.382$, 3 d.f., $P > 0.76$); the interaction also was not significant ($P = 0.27$). *P* values for fourteen unpaired, one-sided *t*-tests of the individual supercooling points of the five butterflies in categories 1–14 when naturally wetted (column C) and then dried by us (column D) are given in column E. These comparisons indicate that the wet butterflies from the respective samples have much warmer supercooling points than when they are dried, and that the lower degree of wetting of the less exposed samples significantly reduces the danger of freezing.

Supercooling points and the amount of surface water on the monarchs

When water was experimentally added to butterflies by spraying (Table 2), a highly significant logarithmic relationship was found between the amount of surface water and the supercooling points: $y = 1.284 (\log x + 0.05) - 5.792$, $r^2 = 0.52$, d.f. = 1, 102, $F = 109$, $P < 0.0001$. (A constant of 0.05 mg of water was added to each mean water weight to obtain positive values.) The data indicate that small amounts of water on a butterfly reduce its cryoprotection, with the effect saturating at about 30 mg. The same analysis applied to the fourteen mean values for the naturally wetted butterflies in Table 1 (regression of values in column C on B) showed a similar logarithmic relationship:

$y = 7.365(\log x + 0.05) - 16.484$, $r^2 = 0.68$, d.f. = 1, 12, $F = 25.9$, $P < 0.0005$, and a similar saturation level at about 30 mg per butterfly. However, the artificially sprayed butterflies began losing their protection with smaller amounts of water on them, probably because their bodies *per se* were more thoroughly wetted than were the naturally wetted butterflies from the clusters.

Fig. 2 summarizes the supercooling points for seventy-six wetted monarchs (those with >1 mg H₂O on their surfaces) from our various 1985 experimental samples and compares them to 410 supercooling points for dry monarchs that were sampled

Table 2. Mean supercooling points (\pm SE) of 104 monarch butterflies collected from the overwintering colony that were experimentally sprayed with water. There is a highly significant logarithmic relationship between the supercooling points (*y*) and the amount of water on the body surface (*x*): $y = 1.284 (\log x + 0.05) - 5.792$; $r^2 = 0.52$; d.f. = 1, 102; $F = 109$; $P < 0.0001$.

	Mean water added (mg)	Mean SCP ($^{\circ}$ C)
Dry (control) (<i>n</i> = 20)	0	-7.7 \pm 0.6
One spray (<i>n</i> = 14)	4.2 \pm 0.7	-5.2 \pm 0.5
Two sprays (<i>n</i> = 21)	16.0 \pm 1.8	-4.4 \pm 0.1
Three sprays (<i>n</i> = 14)	28.2 \pm 1.5	-3.8 \pm 0.2
Four sprays (<i>n</i> = 14)	36.7 \pm 3.3	-3.9 \pm 0.3
Five sprays (<i>n</i> = 7)	44.6 \pm 2.9	-3.7 \pm 0.4
Nine sprays (<i>n</i> = 14)	79.6 \pm 4.2	-4.1 \pm 0.2

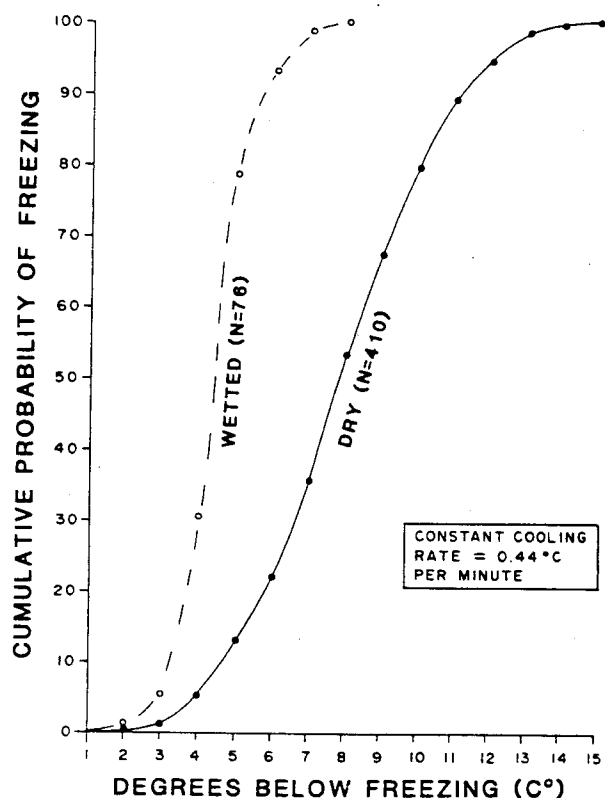


Fig. 2. Experimentally determined freezing probabilities (expressed as the cumulative per cent of the experimental samples) for wet and dry monarch butterflies collected from the Sierra Chincua overwintering site in Mexico. For seventy-six experimentally wetted butterflies (with >1 mg of water on their bodies), 50% mortality occurred at -4.2°C , and 100% mortality occurred at -7.7°C . In contrast, 50% mortality of 410 dry butterflies occurred at -7.7°C and 100% mortality occurred at -15°C .

from the clusters in 1985 and in 1986. At a temperature of -4.2°C , 50% of the wetted butterflies froze and at -8°C , 100% froze. In contrast, 50% of the dry clustering monarchs froze at -7.7°C , and 100% mortality occurred at -15°C . Wetting thus effectively halves the temperature at which monarchs freeze in the experiment.

Canopy cover and rainfall

The nineteen less-exposed quadrats had a mean total rainfall for the three March storms of 31.5 ± 2.0 (SE) ml per cup, significantly less than the twenty more exposed quadrats, with a mean of 44.2 ± 2.3 (SE) ml per cup (unpaired *t*-test, $t = 19.2$, d.f. = 37, $P < 0.001$). These results verified that the canopy functions as a partial rain umbrella for the clustering monarchs.

Exposure and radiational cooling

Monarchs under the most dense glasshouse netting (5% exposure) had body temperatures approximately the same as

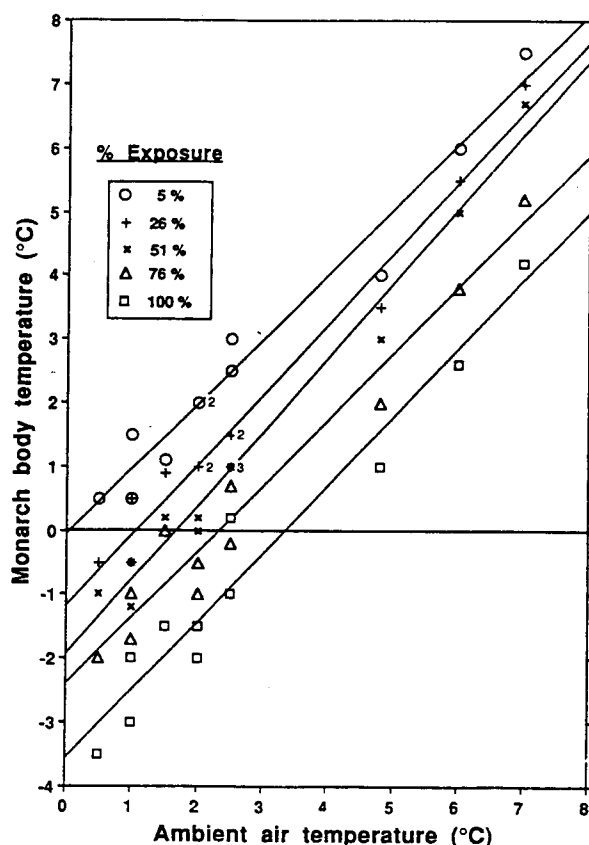


Fig. 3. Ambient air temperatures versus body temperatures of fifty-five monarch butterflies at 04.30 hours measured over several nights at five different levels of experimentally manipulated exposure to the clear night sky. The 5% exposure group was effectively at ambient air temperature, whereas all the other groups fell progressively below ambient temperatures. Note that 100% exposure to the clear night sky depressed the body temperature about 3.5°C below ambient temperatures.

ambient air temperatures (measured at the same height), while the more exposed butterflies had body temperatures below ambient, in direct proportion to the degree of exposure (Fig. 3). Analysis of covariance (Table 3A) indicated that both the percentage of cover and the ambient temperature had highly significant effects on the butterfly body temperatures, but the interaction between the two was not significant. These ANOVA results indicate that the slopes of the five experimental exposure levels in Fig. 3 do not differ, but that their intercepts do. In other words as the parallel lines in Fig. 3 imply, the body temperatures of the monarchs are lowered below the ambient temperatures with increasing degrees of exposure in virtually identical ways under the variable ambient temperatures. Fisher's Protected LSD test indicated that all five body temperature intercepts differed significantly from each other ($P < 0.005$).

Because the slopes of the different exposure lines did not differ, we could run a multiple linear regression analysis to predict the combined effects of ambient temperature and the degree of exposure on the butterfly body temperatures. As shown in Table 3B, the variables are highly correlated ($r^2 = 0.968$, $P < 0.0001$) and relate to each other as follows: $T_{\text{body}} = 1.078$

Table 3. Statistical analyses of the effects of the percentage of exposure on clear nights in decreasing the body temperatures of monarch butterflies below ambient temperatures. (Based on the data in Fig. 3.)

(A) Analysis of covariance

Source of variation	d.f.	SS	MS	F-values	P-values
Per cent exposure	4	26.86	6.71	30.05	0.0001
Ambient temperature	1	272.01	272.01	1217.39	0.0001
Interaction	4	0.627	0.157	0.701	0.5953
Residual	45	10.055	0.223	—	—

(B) Multiple linear regression analysis of the effects of the degree of exposure depressing body temperatures of monarchs exposed to the clear night sky at various ambient temperatures.

Source of variation	d.f.	ANOVA		F-values	P-values	r ²
		SS	MS			
Regression	2	345.23	172.62	785.63	0.0001	0.97
Residual	52	11.43	0.22	—	—	—
Total	54	356.66	—	—	—	—

Regression line: Y (body temperature) = 1.078 (ambient temperature) - 0.034 (percent exposure) - 0.089.

[T_{ambient}] - 0.034 [% exposure] - 0.089. Thus during a clear night that dropped down to -5°C , butterflies that were 100%, 50%, 25% or 5% exposed would have body temperatures of -8.88 , -7.18 , -6.33 and -5.65°C , respectively.

Thus the body temperature of completely exposed dry butterflies on a clear night at an ambient temperature of -5°C would drop to -8.88°C . As can be seen by reading the cumulative probability graph (Fig. 2), mortality of the dry butterflies on a clear night would soar from about 13% to greater than 60%. If the butterflies were wet and the sky cleared at the same ambient temperature of -5°C , the drop in their body temperature to -8.88°C would result in mortality increasing from about 82% to 100%. This scenario was almost realized in at least one of the major overwintering colonies in Mexico during February 1992 (Brower, in Culotta, 1992).

Discussion

The present study of the effects of environmental factors on monarch cold-hardiness clarifies the interactive aspects of several previous observations in the Oyamel forest ecosystem. For example, it was found that over three nights during January 1980, air temperatures at ground level in open areas adjacent to a monarch colony were on average 3.6°C colder than beneath the Oyamel firs within the colony (Calvert & Brower, 1981). Calvert *et al.* (1982) then found that commercial thinning of the Oyamel forest near the Sierra Chincua colony resulted in lower temperatures than in intact forests nearby. In another study carried out during February 1982, Calvert & Cohen (1983) found that the ambient air temperature in an area exposed to the clear night sky was 4.4°C colder on the ground than one meter above the ground. Calvert *et al.* (1982) also found that ground temperature declined with distance from a large fir tree into a clearing: at 12 m from the trunk of tree the temperature was $>4^{\circ}\text{C}$ lower than directly

beneath the tree. As summarized in Geiger (1980), these lower temperatures result from radiational cooling of the exposed substrate to the clear night sky.

Monarch mortality in the Mexico overwintering colonies due to cold temperatures was reported in four previous studies to be greatly increased (and in most cases associated with increased butterfly weight because of dew/frost on their surfaces) when the insects were left on the ground all night in open areas as compared to those left on the ground beneath understorey vegetation (Calvert *et al.*, 1982, 1986), within the monarch colony (Calvert & Brower, 1981), or perched on stakes 1 m above the ground (Calvert & Cohen, 1983). Minimum air temperatures at ground level where much mortality occurred were not drastically low: only -1.7°C on average in one study and -4.9°C in another. Calvert *et al.* (1986) found that 50% of monarchs exposed all night on the ground in open areas died or lost flying ability when ambient air temperatures dropped to -3°C .

Based on our controlled measurements of monarch supercooling points as summarized in Fig. 2, it is clear that the cold mortality previously observed in the butterfly colonies cannot be explained simply by exposure to lower ambient air temperatures. As was predicted by Calvert & Brower (1981), inoculative freezing from dew or frost is a major factor in the lethality of cold. This prediction was field tested at the overwintering site by Alonso-Mejia *et al.* (1992). These authors found a significant reduction in freezing mortality of overwintering monarchs that spend the night above ground and remain dry. Their field results agree closely with our controlled measurements of supercooling points: at below 30 cm perch height they found 50% mortality of dry monarchs at an ambient temperature of -8.0°C , whereas butterflies wetted by dew suffered 50% mortality at an ambient temperature of -4.0°C ; our findings indicated 50% survival at -7.7°C when dry, and -4.2°C when wet.

These previous studies, together with the results in this paper, indicate that (1) the moderate supercooling ability of monarchs is reduced if surface moisture is present on the insects; and (2)

exposure to the clear night sky reduces the butterflies' freeze resistance because their body temperatures drop *below* ambient air temperatures. Wetting and exposure, however, do not occur independently, since exposed butterflies are frequently wetted by winter storms and by dew. By assuming that the effects of wetting and exposure are additive, we generated Fig. 4 to predict their joint impact on butterfly mortality.

Fig. 4, a three-dimensional XYZ plot, was generated using Deltagraph, version 2.0.1 (Wulf *et al.*, 1991). The vertical axis, Z, is the ambient air temperature at which 50% of the monarchs freeze and die. The effect of wetness (mg of water on the body surface) on this mean lethal ambient air temperature is plotted as columns along the Y (front right) axis. The effect of exposure (% exposure to the night sky) on the same mean

lethal ambient air temperature is graphed as columns along the X (front left) axis. For example, butterflies with 30 mg of water on their body, and 20% exposure to the sky, will freeze, on average, when the air temperature drops to -3.6°C ; if the same butterflies were 100% exposed to the sky they would freeze when the air temperature reached -0.8°C , thus losing 2.4°C of cryoprotection.

We derived the values in Fig. 4 as follows: (1) The supercooling assays summarized in Fig. 2 showed that the mean lethal body temperature of 50% of the dry butterflies was approximately -8°C . Since unexposed dry butterflies have $T_{\text{body}} = T_{\text{ambient}} = -8^{\circ}\text{C}$ is also their mean lethal air temperature, and is represented by the flat square at the front of Fig. 4. Both exposure and wetness raise the air temperature at which butterflies freeze, relative to

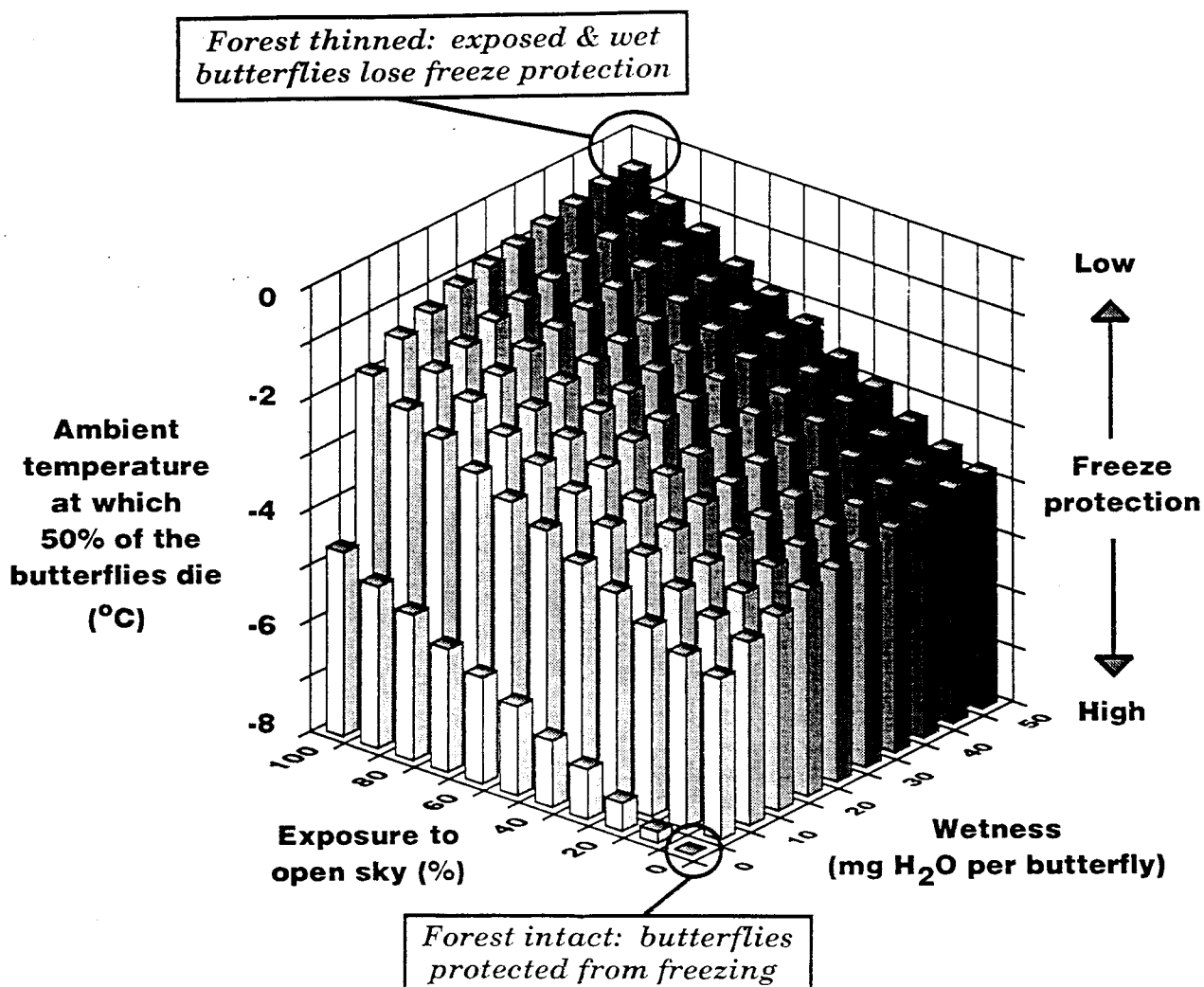


Fig. 4. Surface wetting and increased exposure to the clear sky each increase the probability that individual monarch butterflies will freeze to death; this figure shows their combined effects. The vertical axis indicates the ambient air temperature at which 50% of a sample of butterflies will die. Dry unexposed monarchs (the flat square at the front of the graph) tolerate the lowest ambient temperatures (50% die at -8.0°C), whereas monarchs wetted with 50 mg of water and 100% exposed to the sky (the tallest bar) can tolerate an ambient temperature of only about -0.5°C , effectively losing 7.5°C of their natural cryoprotection. The data indicate how thinning of the forest exacerbates monarch butterfly mortality caused by natural freezing at their high altitude overwintering sites in Mexico. The butterflies are particularly vulnerable on clear nights immediately after wet winter storms that frequently impact the area.

this -8°C minimum. (2) The rightmost row of columns, showing mean lethal air temperature versus wetness at 0% exposure, was determined from the artificial wetting experiments summarized in Table 2. The 104 experimentally determined supercooling points in Fig. 2, ranging from -1.3 to -13.0°C , were regressed against the mg of water on the body surface (x), and a line of best fit to a simple regression model was determined ($y = 1.284 \log(x + 0.05) - 5.972$; $r^2 = 0.52$; d.f. = 103; $F = 109.5$; $P = 0.0001$). This equation was then used to calculate the mean body temperatures at which butterflies wetted with differing amounts of water would freeze. Since these butterflies are unexposed, body temperature equals ambient temperature, which is plotted in the rightmost set of columns. Note that the relationship is logarithmic so that minimal wetting causes an initial jump in raising the freezing temperature. (3) The front row of columns, showing mean lethal air temperature versus variable exposure at 0 mg wetness, was determined from the exposure data in Fig. 3. Since exposure causes body temperature to fall below ambient air temperature, 50% of the dry butterflies will freeze at the combination of ambient temperature and exposure which causes $T_b = -8^{\circ}\text{C}$. Thus, the probable 50% lethal air temperature for the fifty-five butterflies in Fig. 3 was calculated to be $-8^{\circ}\text{C} + (T_a - T_b)$, and these lethal air temperatures (y) were regressed against percent exposure (x). The line of best fit was linear: $y = 0.034x - 8.13$ (d.f. = 1, 53, $F = 302$, $r^2 = 0.851$, $P < 0.0001$), and used to determine the values for the left front row of columns in Fig. 4. (4) The combined effects of wetness and exposure were then estimated by summing their individual effects. For example, 10 mg of water on the body raises the butterflies' freezing temperature by 3.3 degrees, and at 20% exposure the body temperature is 0.5 degrees lower than the ambient air temperature. A butterfly at 20% exposure, with 10 mg of water on its body, therefore has a probable lethal air temperature of $-8^{\circ}\text{C} + (3.3 + 0.5) = -4.2^{\circ}\text{C}$.

Fig. 4, together with the data from Tables 1 and 2, suggests that when overwintering butterflies are wetted with as little as 30 mg of rain and are 100% exposed to the night sky, half of them will die at an air temperature of only -0.8°C (rear, top-most column). This value is 7.2°C warmer than the mean lethal temperature for dry, unexposed butterflies (the bottom, front most column). This degree of wetting can in fact be far exceeded in natural overwintering areas: Calvert *et al.* (1986) found that exposed butterflies on a clear evening in the Sierra Chincua gained an average of 140 mg per butterfly because of frosted dew that formed upon them.

Conclusions

The new data presented in this paper, together with the several previously cited studies of freezing mortality, indicate that shelter both from wetting and exposure is indisputably important in the ability of monarchs to survive at the Mexican overwintering sites. The dangers of freezing probably also have played an important role in shaping the time of the autumn departure from the northern breeding grounds, the time of the spring remigration, and the migratory routes to and from Mexico.

Selective forces resulting from this interplay of cryobiology and the Oyamel forest microclimate system during winter must have strongly favoured the evolution of shelter-seeking behaviours by monarchs at the overwintering sites. Along with predator pressure and facilitation of successful mating, these forces may have contributed both to the dense clustering behaviour, the cluster architecture *per se*, and to monarch's choice of the tight forest canopy cover provided by the Oyamel ecosystem as an overwintering refuge. An intact forest canopy which affords shelter from the night sky and some protection from wetting is clearly critical to monarch survival.

The experiments also determined that the architecture of the butterfly clusters is important in protecting the butterflies from wetting and that individuals inside and on the bottom of the fir bough clusters were better protected from wetting than those on the outside. This supports the hypothesis the structure of the butterfly clusters has evolved, at least in part, through individual selection to avoid wetting.

Finally, our data strongly reinforce the previous contention (Brower & Malcolm, 1991) that the management rationale for the conservation of the monarch's migratory phenomenon must include the prohibition of forest thinning. It is now eminently clear that an intact Oyamel fir forest serves both as a thermal blanket and a rain umbrella for the overwintering monarch butterflies. The final message of this study is that the removal of even a single large tree from an intact forest will result in thousands of monarchs dying during winter storms at the overwintering sites in Mexico. General thinning of the forest, as has recently been proposed by Hoth (1955) and by Chapela *et al.* (1995) would probably result in near total mortality of the colonies during the clearing that follows winter storms.

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