

## Reply to Nylin, Wickman & Wiklund regarding sex ratios of California overwintering monarch butterflies

DENNIS F. FREY & KINGSTON L. H. LEONG

*Biological Sciences Department, Cal Poly State University, San Luis Obispo, CA 93407, U.S.A.*

In an earlier work (Frey & Leong 1993), we (1) determined that roosting height of central coastal California overwintering monarch butterflies, *Danaus plexippus* (L), was independent of sex, (2) determined that males and females were equally 'catchable', thereby eliminating sampling bias as the cause of male-biased sex ratios, and (3) eliminated, on logical grounds, three other widely held hypotheses regarding male-biased sex ratios often reported in Lepidoptera populations (Brussard & Ehrlich 1970). We suggested that male-dominated overwintering populations of western North American monarchs may be attributed to a disproportionate mortality factor between the sexes prior to and during fall migration.

Nylin et al. (1995), drawing from evolutionary stable strategy models on adaptive variation in timing of life-history stages (e.g. Bulmer 1983; Iwasa et al. 1983; Parker & Courtney 1983) and their own work on Pierid butterflies (Wiklund et al. 1992), present an alternative explanation for male-biased ratios among overwintering California monarch butterfly populations. They clearly state that their hypothesis represents a different level of analysis and is therefore not necessarily mutually exclusive of ours (see Holekamp & Sherman 1989 regarding levels of analysis). Our reply here is to (1) clarify several points regarding monarch demographics and clustering phenology and (2) comment on their alternate hypothesis.

Sakai (1991) did an extensive monarch mark-release-recapture census and found male-biased ratios throughout the range of California overwintering sites. His study involved 47 overwintering sites between San Diego and Marin County and 65 585 individuals. During the 1991–1992, 1992–1993 and 1993–1994 seasons at the North Beach campground site, Pismo Beach, California, we recorded male-biased mark-release-recapture sex ratios throughout each overwintering season, suggesting that male-biased ratios were both

spatially and temporally pervasive (Leong & Frey 1992; unpublished data).

Nylin et al. (1995) propose that in late summer and early autumn monarch populations, males should have 'a greater propensity to enter diapause development, and accordingly be over-represented among overwintering individuals'. Their evolutionary stable strategy-based model further implies that males ought to initiate migration to overwintering sites earlier than females. We found neither a pattern of greater male bias during the early phase (autumn) of colony increase nor a decline in male relative abundance at peak population levels, which normally occur in late December (Table I). James (1984) reported similar data for two overwintering seasons at a site in Camden, New South Wales, Australia. Selective advantage of early autumn arrival by males (relative to females) at overwintering sites is questionable because the critical aspects of evolutionary stable strategy life-history theory is focused on the timing of diapause emergence prior to and/or during the reproductive phase (February to early March in the case of *D. plexippus* (L) at central coastal California overwintering sites).

The model of Nylin et al. (1995) also assumes that the earlier that males enter diapause, the sooner they will become sexually mature in the spring to maximize the number of matings. Their model does not, however, account for the state of reproductive diapause nor, once diapause is broken, the rate at which reproductive development occurs between the sexes. We have dissected field-collected monarchs ( $N=20$  males and 20 females) twice monthly, between 18 October 1990 and 22 March 1991 (K. L. H. Leong, unpublished data). The microscopic examination of female reproductive tracts for egg development revealed that they remained undeveloped until the second week of January, when 20% of the females showed signs of ovarian development (i.e. increase in egg

**Table I.** Seasonal sex ratio (% male) of overwintering monarchs at North Beach campground, Pismo Beach, California

Time	Season		
	1990–1991	1991–1992	1992–1993
Early November	59.4 ( <i>N</i> =900)	67.2 ( <i>N</i> =1000)	53.7 ( <i>N</i> =1400)
Late November	61.4 ( <i>N</i> =1000)	66.0 ( <i>N</i> =1000)	58.0 ( <i>N</i> =1400)
Late December	62.3 ( <i>N</i> =1164)	67.0 ( <i>N</i> =1105)	56.1 ( <i>N</i> =353)
Late February	87.0 ( <i>N</i> =431)	86.2 ( <i>N</i> =370)	73.1 ( <i>N</i> =283)

diameter within the ovaries). The number of females exhibiting oogenesis increased steadily until 100% of the females in March samples had developing eggs within their ovaries. In contrast, we observed active spermatozoans from the male testes throughout the winter months, a condition also reported by Hill et al. (1976). The difference in reproductive readiness (development) agrees with Herman's (1981) conclusion that female monarchs had more intense and longer duration diapause than the males. Our data suggest that overwintering males, because of the presence of spermatozoans within their testes, are capable of becoming sexually mature sooner than the females, even if both sexes were to resume reproductive development at the same point in time. In addition, the female's mating activity is not indicative of ovarian development, because approximately 40% of the diapausing females arriving in autumn had spermatophores in their bursa copulatrix and spermatozoans in their spermatheca. These females may have mated with non-diapausing summer generation males, similar to females of *Eurema hecabe* reported by Kato (1989). The timing of oogenesis in spring/summer generation monarchs has also been shown to be independent of mating (K. Oberhauser, personal communication). The % of mated females at our sites remained statistically unchanged until a few weeks prior to their spring dispersal.

Females tend to disperse earlier than males at California overwintering sites. Supporting evidence includes: (1) male-biased sex ratios become even more extreme during the final weeks of overwintering (Table I; see also Hill et al. 1976; Tuskes & Brower 1978) and (2) in a study by Nagano et al. (1993), marked females were

recovered with greater than expected frequency along California spring migration routes, whereas males exhibited the opposite pattern (our analysis of data in Table II of their study: 18 216 females marked, 32 555 males marked, 46 females recaptured, 54 males recaptured;  $\chi^2=4.44$ ,  $P=0.035$ ). A number of adaptive explanations can account for this pattern, however. Extensive wing damage, for example, is incurred during the period of intense mating activity, which normally occurs during the last several weeks of overwintering (Leong et al. 1993). Early departure by females, therefore, probably minimizes their wing damage and increases their dispersal range. Females typically mate several times during the relatively brief mating phase at California overwintering sites, and their early departure may reduce other costs associated with 'overmating' such as ruptured bursa copulatrix (Oberhauser 1989; personal observation).

It is believed that female monarchs use male-derived accessory gland nutrients from spermatophores for somatic maintenance and/or egg development (Boggs & Gilbert 1979; Boggs 1981; Oberhauser 1989; Wells et al. 1993). These nutrients may be particularly advantageous after months of overwintering, when body fat reserves are low. For overwintering monarchs, the timing of emergence from diapause relative to the timing of spring dispersal is subject to constraints on reproductive development (e.g. influence of temperature and photoperiod), rapid demographic transitions, and selective pressures on mating activity (e.g. costs and benefits of multiple mating). These attributes of the biology of the monarch are not factored into the model proposed by Nylin et al. (1995).

Whereas Nylin et al. present an 'ultimate, adaptive, explanation following directly from evolutionary stable strategy models', they invoke an additional proximate level of explanation by extensive reference to Lessman & Herman's (1983) study on the role of juvenile hormone in monarch sexual maturation and mating activity. Nylin et al. contend that Lessman & Herman's reported pattern of juvenile hormone haemolymph titre change (see Figure 2 in Lessman & Herman 1983), and the pattern of late summer/early autumn mating frequency, support their 'propensity to diapause' hypothesis. Their interpretation of Lessman & Herman's results is tenuous at best, because that study lacks critical tests of statistical significance. Similarly, the pattern of mating frequency reported in Lessman & Herman (i.e. August, high; September, low; October, high) has not been reported for western North American monarch populations.

In summary, Nylin et al. (1995) offer a possible mechanism for the male-biased sex ratios observed for the western North American monarch overwintering populations. We suggest, however, that their model is more applicable to insects with discrete generations (e.g. the Pierids from which most of their generalizations are derived) than for multivoltine organisms like monarchs with overlapping generations (Cockrell et al. 1993). Their model also (1) fails to fit seasonal sex-ratio patterns for California overwintering monarch populations, (2) does not address migrational components, (3) discounts variation in the degree of diapause and relative rates of sexual maturation during diapause emergence, and (4) overlooks many temporal aspects of monarch reproductive strategies at overwintering sites.

Our research was supported by grants from California Polytechnic State University and California Department of Parks and Recreation.

## REFERENCES

- Boggs, C. 1981. Selection pressure affecting male nutrient investment at mating in heliconiine butterflies. *Evolution*, **35**, 931-940.
- Boggs, C. & Gilbert, L. 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science*, **206**, 83-84.
- Brussard, P. & Ehrlich, R. 1970. The population structure of *Erebia epipsodea* (Lepidoptera: Satyriinae). *Ecology*, **51**, 119-129.
- Bulmer, M. 1983. Models for the evolution of protandry in insects. *Theor. Pop. Biol.*, **23**, 314-322.
- Cockrell, B., Malcolm, S. & Brower, L. 1993. Time, temperature, and latitudinal constraints on the annual recolonization of eastern North America by the monarch butterfly. In: *Biology and Conservation of the Monarch Butterfly* (Ed. by S. Malcolm & M. Zalucki), pp. 233-252. Los Angeles: Natural History Museum of Los Angeles County.
- Frey, D. & Leong, K. L. H. 1993. Can microhabitat selection or differences in 'catchability' explain male-biased sex ratios in overwintering populations of monarch butterflies? *Anim. Behav.*, **45**, 1025-1027.
- Herman, W. 1981. Studies on the adult reproductive diapause of the monarch butterfly, *Danaus plexippus*. *Biol. Bull.*, **160**, 89-106.
- Hill, H., Wenner, A. & Wells, P. 1976. Reproductive behavior in an overwintering aggregation of monarch butterflies. *Am. Midl. Nat.*, **95**, 10-19.
- Holekamp, K. & Sherman, P. 1989. Why male ground squirrels disperse. *Am. Scient.*, **77**, 232-239.
- Iwasa, Y., Odendal, F., Murphy, D., Ehrlich, P. & Launer, A. 1983. Emergence patterns in male butterflies: a hypothesis and a test. *Theor. Pop. Biol.*, **23**, 363-379.
- James, D. 1984. Population and general biology of non-reproductive colonies of the monarch butterfly, *Danaus plexippus* (L) (Lepidoptera: Danaidae) in New South Wales. *Austral. J. Zool.*, **32**, 663-670.
- Kato, Y. 1989. Differences in reproductive behavior among seasonal wing morphs of the butterfly *Eurema hecabe*. *J. Insect Behav.*, **2**, 419-429.
- Leong, K. L. H. & Frey, D. 1992. Monarch butterfly winter habitat study. Final Report to California Department of Parks and Recreation.
- Leong, K. L. H., Frey, D., Hamaoka, D. & Honma, K. 1993. Wing damage in overwintering populations of monarch butterfly at two California sites. *Ann. entomol. Soc. Am.*, **86**, 728-733.
- Lessman, C. & Herman, W. 1983. Seasonal variation in hemolymph juvenile hormone of adult monarchs (*Danaus plexippus*: Lepidoptera). *Can. J. Zool.*, **61**, 88-94.
- Nagano, C., Sakai, W., Malcolm, S., Cockrell, B., Donahue, J. & Brower, L. 1993. Spring migration of monarch butterflies in California. In: *Biology and Conservation of the Monarch Butterfly* (Ed. by S. Malcolm & M. Zalucki), pp. 219-232. Los Angeles: Natural History Museum of Los Angeles County.
- Nylin, S., Wickman, P.-O. & Wiklund, C. 1995. An adaptive explanation for male-biased sex ratios in overwintering monarch butterflies. *Anim. Behav.*, **49**, 511-514.
- Oberhauser, K. 1989. Effects of spermatophores on male and female monarch butterfly reproductive success. *Behav. Ecol. Sociobiol.*, **25**, 237-246.
- Parker, G. & Courtney, S. 1983. Seasonal incidence: adaptive variation in the timing of life history stages. *J. theor. Biol.*, **105**, 147-155.
- Sakai, W. 1991. Monarch sabbatical update. *Monarch Newsl.*, **2**, 4-5.

- Tuskes, P. & Brower, L. 1978. Overwintering ecology of the monarch butterfly, *Danaus plexippus* (L), in California. *Ecol. Entomol.*, **3**, 141-153.
- Wells, H., Wells, P. & Rogers, S. 1993. Is multiple mating an adaptive feature of monarch butterfly winter aggregations? In: *Biology and Conservation of the Monarch Butterfly* (Ed. by S. Malcolm & M. Zalucki), pp. 61-68. Los Angeles: Natural History Museum of Los Angeles County.
- Wiklund, C., Wickman, P.-O. & Nylin, S. 1992. A sex difference in the propensity to enter direct/diapause development: a result of selection for protandry. *Evolution*, **46**, 519-528.