sistent with the hypothesis of a hormonal factor in the etiology of cervical cancer. This concept is of scientific interest since the uterine cervix is an endocrine target organ—the structure and function of the mucosa is responsive to the internal hormonal environment, and specific estrogen-binding proteins have been found in the human cervix (*15*). Some insight into the biological mechanism by which the contraceptive pill as a synthetic steroidal progestin-estrogen compound could exert an adverse effect on cervical mucosa is provided from experimental work on competition by synthetic hormones for specific protein receptor sites in cervicovaginal epithelium (*16*). Further investigation of the cervix as a target organ may provide a rationale for considering hormone dependency as a factor in the management of advanced cervical cancer.

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References and Notes

1. E. Stern, *Cancer Res.* **33**, 1368 (1973).
2. D. Seigel and P. Corfman, *J. Am. Med. Assoc.* **203**, 950 (1968).
3. G. Pincus, *The Control of Fertility* (Academic Press, New York, 1965), pp. 253–259; C. M. Dougherty, *Obstet. Gynecol.* **36**, 741 (1970); A. J. Worth and D. A. Boyes, *J. Obstet. Gynaecol. Br. Commonw.* **79**, 673 (1972); D. B. Thomas, *Obstet. Gynecol.* **40**, 508 (1972); H. Ory, Z. Naib, S. B. Conger, R. A. Hatcher, C. W. Tyler, Jr., *Am. J. Obstet. Gynecol.* **124**, 573 (1976).
4. M. R. Melamed, L. G. Koss, B. J. Flehinger, R. P. Kelisky, H. Dubrow, *Br. Med. J.* **3**, 195 (1969).
5. M. R. Melamed and B. J. Flehinger, *Gynecol. Oncol.* **1**, 290 (1973).
6. E. Stern, V. A. Clark, C. F. Coffelt, *Science* **169**, 497 (1970); E. Stern, P. Shankman, C. F. Coffelt, L. Youkeles, A. Forsythe, *Contraception* **7**, 435 (1973).
7. Evaluation of concomitant variables was based on the study of characteristics that distinguish subjects with dysplasia and normal subjects before the selection of contraceptive method; characteristics that distinguish between subjects who chose the pill and subjects who chose other methods of contraception, before use of the pill; a profile of reasons for dropping out of the study in relation to duration in the study and contraceptive method; and characteristics that distinguish women who drop out from those who stay in the study at each time of follow-up. In order to adjust for the possible bias of self-selection, characteristics related to contraceptive group and to disease outcome were identified. The set used as covariates is age; number of pregnancies; cytohormonal variables; baseline score; and the classic risk factors of age at first intercourse, race, and education. Only two of these variables—age at entry into the study and number of previous pregnancies—were significantly related to contraceptive group, subjects taking the pill being about 3 years younger and having one less pregnancy than those not taking the pill, on the average.
   To ensure against bias by failure to include in the set of covariates any factor related to differential dropout between pill users and nonusers, we analyzed the characteristics of subjects continuing in the study and those dropping out. Covariance analysis was applied at each of 12 time intervals to three contrasts: method (pill users versus nonusers), follow-up status (dropping out versus staying in), and method by follow-up interaction. The demographic and biomedical variables examined were adjusted by the above set of identified risk factors. We found no evidence that subjects dropping out of the study introduced bias into the results on the basis of the variables considered. In particular, there was no significant difference in the dysplasia score at each time interval between subjects dropping out and subjects staying in, nor was there a significant interaction between dropout status and contraceptive method. However, we cannot rule out the possible effect of unmeasured or unmeasurable variables.
   Covariate adjustment did not change the results shown in the sample of normal subjects. In dysplasia, differences between pill users and nonusers are increased with covariate adjustment, becoming statistically significant at 2, 3, and 4 years of follow-up. Covariate adjustment did not alter the significant differences observed between pill users and nonusers in nonreverters. The adjusted analyses indicate that it is unlikely that the substantive results of this study are a consequence of the subjects' initial self-selection of the pill or other method of contraception or of differential dropout.
8. Demographic features of the base population included a racial composition of 47 percent black, 38 percent Mexican-American, 13 percent white, and 2 percent others. Ages ranged from 14 to 49; approximately 5 percent were over 35, and the median age was about 21. The number of births ranged from 0 to 12; 13 percent had not yet borne a child and 34 percent had only one birth. The median monthly income was about $400 per household.
9. L. Youkeles, A. B. Forsythe, E. Stern, *Cancer Res.* **36**, 2080 (1976).
10. The steroid contraceptive was restricted to one compound, Ovulen (ethynodiol diacetate, 1 mg, with mestranol, 0.1 mg), for 21 days of the 28-day regimen. All types of intrauterine devices were available. Diaphragm, foam, rhythm, or condom were the contraceptive methods for a small number of women. During the study, 66 percent of the subjects with dysplasia and 59 percent of the normal subjects used Ovulen.
11. B. A. Barron and R. M. Richart, *J. Natl. Cancer Inst.* **41**, 1343 (1968).
12. E. Stern, A. B. Forsythe, L. Youkeles, W. J. Dixon, *Cancer Res.* **34**, 2358 (1974).
13. For the detailed analysis of the data see E. Stern, "Effects of Steroid Contraception on Dysplasia," final report for NIH, CHHD, Contract NO1-3-2731, 23 February 1976, pp. 1–73; information may also be obtained from the authors.
14. S. J. Cutler and F. Ederer, *J. Chronic Dis.* **8**, 699 (1958).
15. B. M. Sanborn, B. Held, H. S. Kuo, *J. Steroid Biochem.* **6**, 1107 (1975).
16. W. A. Anderson and Y. H. Kang, *Am. J. Anat.* **144**, 1977 (1975).
17. We thank the staff of the County of Los Angeles Community Health Services for their cooperation, and J. Wargin for his help in maintaining the clinical aspects of this study. W. J. Dixon provided advice throughout the study. We thank P. Smedberg for help with the statistical analyses. Assistance was obtained from the Health Sciences Computing Facility, University of California, Los Angeles, sponsored by NIH grant RR-3. This research was supported by the National Institute of Child Health and Human Development.

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Rapid Response to Selection for a Nondiapausing Gypsy Moth

Abstract. *By genetic selection a gypsy moth strain was obtained within eight generations that could be reared continuously in the insectary without its normal "obligatory" diapause. This strain should be useful for making physiological and genetical comparisons with normal diapausing gypsy moths. It could also be used in a genetic control program. However, caution should be exercised, for its release could create a new environmental hazard.*

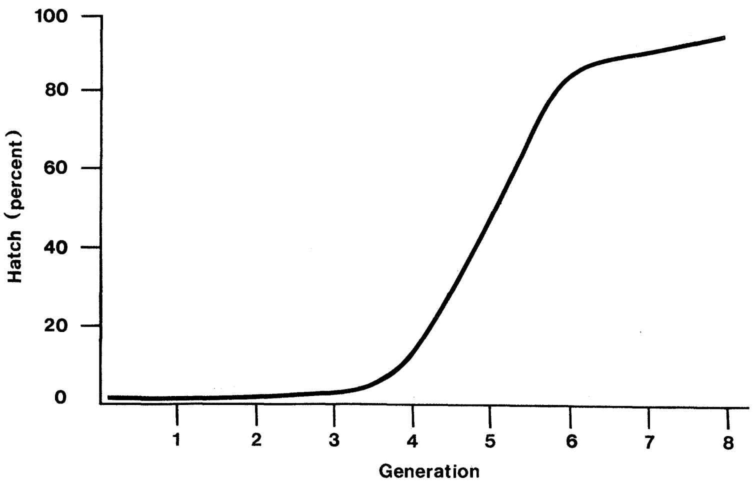
Selection and inbreeding over eight generations have produced a new nondiapausing strain of the gypsy moth, *Lymantria dispar* L. This colony might prove useful in the genetic control of gypsy moths, and shows that it is possible to rear healthy gypsy moths throughout the year for laboratory experiments.

Laboratory research with the gypsy moth is hampered by the fact that the eggs require a minimum of 90 days exposure to a low temperature for termination of the "obligatory" egg diapause (*1, 2*). Consequently, eggs collected in the field for laboratory experiments or insectary production will not hatch for several months each year. Furthermore, the contamination of field-collected eggs with nuclear polyhedrosis virus (NPV) is a problem that surface disinfection does not adequately solve. Thus a virus-free nondiapausing strain would be useful for the mass-rearing of gypsy moth parasites or NPV, or for various experiments including future comparative physiological analyses of diapause and diapause genetics.

It is well known that the duration of diapause in the gypsy moth is variable (*3–5*), but attempts to obtain a nondiapausing strain by genetic selection have not been reported.

Selection for the nondiapausing strain reported here began in October 1973, when 230,000 eggs were collected in the field (in northern Connecticut), disinfected, and pooled. Half of the eggs were held under ambient conditions (22°C, natural daylength) and half were chilled (5°C) for 2 weeks. Larvae were reared on a synthetic diet under controlled constant temperature (22°C) and a long day (18 hours), although previous authors (*2, 6*) reported the gypsy moth to be one of the few insect species that is photoperiodically neutral. The rarity of truly neutral species led me to control the photoperiod. During the F_2 through F_4 generations, "token" chills were given half of the eggs for 2 weeks, to increase the level of hatch. Virus incidence was so low that disinfection of the eggs was not required after the F_1 generation because care was taken to prevent contamination.

The response to selection for rapid



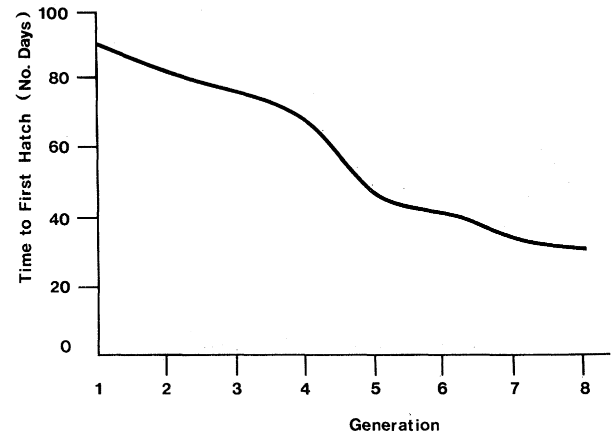


Fig. 1 (left). The percentage hatch of embryonated gypsy moth eggs after no chill (F_4 to F_8), or no chill and 2 weeks of chill (5°C) in the F_1 to F_3 generations. Gypsy moth larvae were reared at 22°C and were subjected to photoperiods of 18 hours of light and 6 hours of darkness. Fig. 2 (right). The mean number of days until the first hatch occurred in each generation. All larvae in the F_1 to F_4 generations were reared, but purposeful selection for earlier hatching began in the F_4 generation. About 14 to 30 days are required for the gypsy moth egg to embryonate so that hatching can begin.

hatching without the necessity for exposure to a low temperature was slow through the first four generations (Fig. 1), but it accelerated in the F_4. The rate of hatching increased from 0.9 percent in F_1 to 56 percent in the F_5. By the F_6 and F_8 it reached 79 and 95 percent, respectively.

During the F_2 through F_4 generations, siblings were mated and outcrosses were made between moths from different egg masses. In subsequent generations only outcrosses between different egg masses were made in order to slow the rate of inbreeding. During the F_1 to F_4 generations, all larvae hatching were reared, but thereafter selection for rapid hatching began.

During the F_5, the first 75 larvae from 87 egg masses that began to hatch within 75 days of egg-mass deposition were reared. During the F_6, the first 75 larvae from 147 egg masses that began hatching within 60 days were reared. In the F_7, the first 50 larvae from 150 egg masses beginning to hatch within 40 days were reared.

Selection greatly reduced the interval from egg-mass deposition to initiation of hatching (Fig. 2). The first generation required more than 90 days before hatching began. This interval declined to 31.5 days by the F_8. Since the gypsy moth is thought to require 14 to 30 days for embryonation (*1, 3, 7*), it may not be possible to select effectively for larvae hatching in much shorter periods than those obtained here. Thus, capacity to hatch without exposure to low temperatures was increased, and the interval until hatching began was substantially decreased.

I therefore suggest that variability in the wild population was present for diapause termination without the requisite chill. The variability yielded the initial 0.9 percent hatch more than 90 days after egg-mass deposition. Subsequent selection of egg masses that began hatching before 90 days may have selected for the rapid termination of a diapause requiring no chilling for its termination. Many insects are variable in their rate of diapause termination (*2*). Aberrant hatching of a few gypsy moth eggs under field conditions has been widely reported (*5, 6*).

Recent changes in voltinism of another pest insect (*8*) suggest that this new gypsy moth colony may be a double-edged sword. On the one hand, it may provide an opportunity to develop a new genetic control for the gypsy moth, although much must be done before this can be ascertained. Previously proposed genetic control methods are limited by the lowered viability of irradiated gypsy moths (*9*); and the production of sterile intersexes from crosses of different geographical races of the gypsy moth carries the risk that a "new and more successful form" might arise (*10*).

A comparable concern may be appropriate with this nondiapausing gypsy moth strain. Accordingly, releases, purposeful or inadvertent, must be precluded until studies can be conducted on the cold-hardiness of the selected strain and of hybrids between it and the wild type, on the phenologies of these insects, their capacity to survive on a natural foliage diet, and on the mode of inheritance of the nondiapause trait. The southern and western dispersal of the broadly polyphagous gypsy moth is likely (*11*).

The selected strain does not seem to have lost all cold tolerance. When F_5 egg masses were stored at 5°C, 70 percent retained the ability to hatch after 5 months. This fact makes the release of this colony dangerous unless or until cold tolerance can be removed through selection. Large-scale introgression of the nondiapause genes into populations spreading south raises the specter of a gypsy moth able to develop continuously and able to devour refoliating trees nearly the year around.

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References and Notes

1. S. Masaki, *Jpn. J. Appl. Zool.* **21**, 148 (1956).
2. A. S. Danilevski, *Photoperiodism and Seasonal Development of Insects* (Oliver & Boyd, London, 1965), p. 39.
3. R. Goldschmidt, *Bibliogr. Genet.* **11**, 1 (1934).
4. K. E. Schedl, *Monogr. Angew. Entomol.* **12**, 1 (1936).
5. E. H. Forbush and C. H. Fernald, *The Gypsy Moth* (Wright & Potter, Boston, 1896). On page 295 Fernald discusses the production of a second brood of larvae in Massachusetts in 1895, noting that the larvae "fed with great avidity" on the "more delicate foliage" of refoliated trees until they were killed by cold.
6. D. E. Leonard, *J. Econ. Entomol.* **61**, 596 (1968).
7. M. Maksimovic, *Posebna Izd. Biol. Inst. N.R. Srb. Beograd.* 3, 1 (1958).
8. W. B. Showers *et al.*, *Ann. Entomol. Soc. Am.* **64**, 1369 (1971). The European corn borer has developed several races since its introduction into North America, and the barrier to southern spread appears to have dropped through adaptation of the diapause. The codling moth has also developed a race that feeds on walnut and another that feeds on plum; both of these races exhibit significantly different induction, termination of diapause, and developmental heat requirements since 1873 in California [P. A. Phillips and H. M. Barnes, *ibid.* **68**, 1053 (1975)].
9. P. A. Godwin, H. D. Rule, W. E. Waters, *J. Econ. Entomol.* **57**, 986 (1964).
10. J. A. Downes, *Can. Entomol.* **92**, 661 (1960).
11. C. R. Sullivan and D. R. Wallace, *ibid.* **104**, 1349 (1972).
12. I thank R. Weseloh, J. F. Anderson, and W. E. Wallner for critical review of the manuscript and P. Derderian, N. Knop, D. Brown, and J. Germe for expert rearing assistance.
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