sum test (*12*), cervical cancer patients (group A) differed significantly from normal donors (group B, $P < .01$) and from patients with other types of cancer (group C, $P < .05$). Group B and group C were not significantly different.

To investigate the possibility that leukocyte migration inhibition may be a result of hCG-β in the CaSki antigen preparations (see below), leukocytes from cervical cancer patients or healthy donors were incubated with highly purified hCG-β (*13*). No inhibition of leukocyte migration was seen with hCG-β at levels comparable to those present in antigen preparations or in patient's serum (not shown). Therefore, it appears that CaSki cells express tumor-associated antigen that can be recovered in concentrates of the culture fluid.

For hormone assays, 5-day double-antibody radioimmunoassays were used (*14*). Concentrates of CaSki culture fluid behaved as highly purified hCG-β when assayed in either the homologous hCG (Fig. 4) or hCG-β (Fig. 5) radioimmunoassays. This indicated that the CaSki cells secreted a substance antigenically identical to hCG-β. The CaSki fluid gave a negative response when assayed in the homologous hCG-α radioimmunoassay (results not included). On the basis of the relative response in the homologous hCG and hCG-β assays, it was determined that the CaSki fluid contained 1 percent or less intact hCG. Gel filtration analysis of the CaSki concentrate (Fig. 6D) resulted in a discrete hCG-β peak that eluted earlier (by one tube) than hCG-β (Fig. 6B) or ^{125}I-labeled hCG-β (Fig. 6C). Similar results were obtained when unconcentrated CaSki culture fluids were analyzed by gel filtration and radioimmunoassay (not shown). The lesser hCG immunoreactivity could be accounted for completely on the basis of the 15 percent cross-reactivity of hCG-β in the homologous hCG radioimmunoassay. The slightly lower elution volume of the CaSki hCG-β peak, as compared to highly purified hCG-β, may reflect minor differences in carbohydrate or amino acid content in the glycoprotein secreted by the CaSki cells.

The presence of hCG (or hCG-β) in the serum of the patient from whom the CaSki cell line was derived, and the production of hCG-β by the tumor cells in culture more than 1½ years later, gives evidence of the apparent irreversibility of the depression that occurred in the production by cervical carcinoma cells of this placental hormone marker. The continuing production of tumor-associated antigen and hCG-β by the tumor cells provides possible tools for the immunodiagnosis and immunotherapy of cervical cancer and for the study of hCG-β synthesis and regulation in malignancy.

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References and Notes

1. N. Zambchek and G. Pusztaszeri, *Cancer J. Clin.* **25**(4), 204 (1975).
2. A. Hertig, *The Human Trophoblast* (Thomas, Springfield, Ill., 1968), p. 24.
3. B. B. Saxena, S. H. Hasan, F. Haour, M. Schmidt-Gollwitzer, *Science* **184**, 793 (1974).
4. R. A. Pattillo, in *Pathobiology Annual*, H. L. Ioachim, Ed. (Appleton-Century-Crofts, New York, 1973), p. 241.
5. S. W. Rosen, B. D. Weintraub, J. L. Vaitukaitis, H. H. Sussman, J. M. Hershman, F. M. Muggia, *Ann. Intern. Med.* **82**, 71 (1975).
6. A. S. Rabson, S. W. Rosen, A. H. Tashjian, Jr., B. D. Weintraub, *J. Natl. Cancer Inst.* **50**, 669 (1973).
7. N. K. Ghosh and R. P. Cox, *Nature (London)* **259**, 416 (1976).
8. Courtesy of Drs. Ward D. Peterson and Walter Nelson-Rees.
9. V. I. Oyama and H. Eagle, *Proc. Soc. Exp. Biol. Med.* **91**, 305 (1956).
10. L. M. Patt and W. U. Grimes, *J. Biol. Chem.* **249**, 4157 (1974).
11. J. L. McCoy, L. F. Jerome, J. H. Dean, G. B. Cannon, T. C. Alford, T. Doering, R. B. Herberman, *J. Natl. Cancer Inst.* **53**, 11 (1974).
12. G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, 1971).
13. We thank O. P. Bahl for his gift of highly purified hCG-β.
14. A. R. Midgley, *Endocrinology* **79**, 10 (1966); D. Rodbard, *Clin. Chem. (N.Y.)* **20**, 1255 (1974).

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Interspecific Hybridization and Caste Specificity of Protein in Fire Ant

Abstract. *One natural population of fire ant in Texas was found to be a hybrid between* Solenopsis geminata *and* S. xyloni. *Evidence from isozyme studies and breeding experiments is provided to demonstrate interspecific hybridization in ants. In this hybrid population, all worker ants have both parental types of nicotinamide adenine dinucleotide–malate dehydrogenase isozymes, but 95 percent of queens possess only the maternal type.*

In the course of taxonomic studies, students of ants often encounter intermediate variants that might represent interspecific hybrids (*1*, *2*). In most cases, these conclusions are based on morphological characters and, at most, are supported by statistical analysis (*2*). Although Cupp *et al.* (*3*) could produce hybrids of *Solenopsis invicta* and *S. richteri* in a study of forced copulation, their results do not demonstrate that hybridization occurs between natural populations of these two imported fire ant species. We now report another case of interspecific hybridization, in nature, between two ant species, but provide, for the first time to our knowledge, evidence from isozyme studies and breeding experiments. We further demonstrate a case of differential expression of parental genes in different castes of ants.

We have been studying the nicotinamide adenine dinucleotide–malate dehydrogenase (NAD–MDH) isozymes of North American fire ants by means of horizontal starch gel electrophoresis. The banding patterns of this enzyme are monomorphic and species-specific in all North American fire ant species (Fig. 1, samples 1 to 9) (*4*). In the summer of

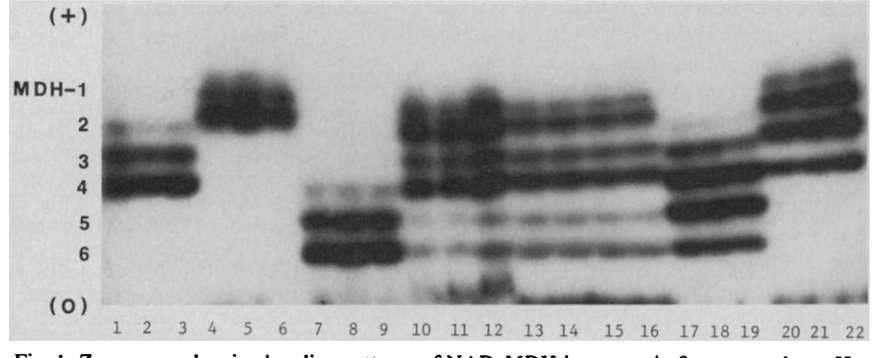

Fig. 1. Zymogram showing banding patterns of NAD–MDH isozymes in fire ant workers. Hybrid samples (10 to 16) have MDH-3 that is not found in parental species. Samples 1 to 3, *S. invicta*; samples 4 to 6, *S. geminata*; samples 7 to 9, *S. xyloni*; samples 10 to 12, *xyloni* × *geminata*, natural; samples 13 to 16, *xyloni* × *geminata*, in vitro; samples 17 to 19, *xyloni* × *invicta*, in vitro; and samples 20 to 22, *invicta* × *geminata*, in vitro.

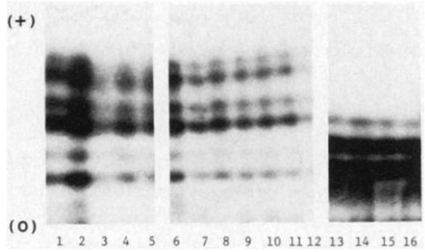

Fig. 2. Zymogram showing banding patterns of NAD–MDH isozymes in larvae of a *S. xyloni* × *geminata* hybrid. Samples 1 to 5, workers, artificial; samples 6 to 12, workers, natural; samples 13 to 16, queens, natural. (Samples 6 to 16 are progeny of the same nest queen.)

1975, we found workers of one natural population of fire ant in College Station, Texas; this population has a unique six-band NAD–MDH isozyme pattern not yet found in any fire ant species (Fig. 1, samples 10 to 12). Comparison of this new pattern with those of the three species of fire ants (*S. geminata*, *S. invicta*, and *S. xyloni*) found in the same area suggests that this population might be a hybrid between *S. geminata* and *S. xyloni*. Recombination in vitro of NAD–MDH subunits in mixed homogenates from workers of two different species (*5*) supported the hybrid hypothesis, because only mixtures of samples of *S. xyloni* and *S. geminata* yielded six bands on electrophoresis (Fig. 1, samples 13 to 22). Further evidence of the hybrid nature of this population was finally obtained when we artificially inseminated virgin queens of *S. xyloni* with males of *S. geminata* and found that the NAD–MDH isozyme banding pattern of five worker larvae produced from one of these crosses was identical to that of the worker larvae from this putative hybrid population (Fig. 2, samples 1 to 12) (*6*).

In insects as well as in vertebrates, the isozyme banding patterns of many enzymes generally change during development (*7*). In interspecific crosses, maternal transmission of enzymes has been reported (*8*, *9*). In the progeny of a cross between the *Drosophila melanogaster* female and the *D. simulans* male, all the aldehyde oxidase present in the egg is of the maternal type. Only much later during the life cycle is the paternal type expressed (*8*). We did not find this type of stage specificity in the NAD–MDH isozymes of the hybrid population. However, we found that, while NAD–MDH isozyme banding pattern of all workers was of the hybrid type, that of most queens (virgin and mated) was of the *xyloni* type (Table 1). This differential expression of parental genes was found even between workers and virgin queens produced by the same nest queen and was detected as early as the larval instar at which a size difference between worker and queen larvae can be recognized (Fig. 2, samples 6 to 16).

In ants, as in other Hymenoptera insects, males are haploid and females (workers and queens) diploid. Workers are ordinarily sterile females with a greatly simplified thorax and reduced ovarioles, whereas queens are winged and fertile. An ant colony usually produces only workers. Under natural conditions, each species has a particular season during which males and virgin queens are reared. Although worker and queen castes in ants are not considered to be genetically determined, several environmental and physiological factors are involved (*10*). We have noticed in fire ants that, when a laboratory colony that has been producing only workers begins to rear queen and male brood, the nest queen of that colony usually dies within a month. We can also induce the production of queen brood in a worker-rearing colony by removing the nest queen before her brood reaches a certain developmental stage and then feeding this queenless colony with insects such as larvae and pupae of cotton bollworms (*Heliothis* spp). Therefore, in fire ants as in other ants, queen influence and larval nutrition are important factors in caste determination (*10*); and there are plastic periods in which some brood are capable of developing into either queens or workers (*11*). In this interspecific hybrid population, we collected 34 virgin queens from two laboratory colonies after nest queens had died and 9 from one colony from which we had removed the nest queen. All 43 queens had only the *xyloni* type NAD–MDH isozymes, but their sister workers were still hybrid in their banding pattern. This indicates that the paternal genes of these 43 female ants were suppressed when they developed into the queen caste. Therefore, the absence of *geminata* type NAD–MDH isozymes in 95 percent of the queens studied suggests an influence of physiological state on the expression of parental genes in this interspecific hybrid. A worker-queen intercaste has been found in *S. invicta* (*12*). The six queens that showed the hybrid pattern were probably also worker-queen intercastes which we misclassified as queens. The hybrid pattern might indicate that their physiological state was tilted more toward the worker caste.

The NAD–MDH isozyme banding pattern of all 39 males studied was of the *xyloni* type and that of all workers was of the hybrid type (Table 1). These patterns

Table 1. Frequency of NAD–MDH isozyme banding patterns in 16 *Solenopsis xyloni* × *geminata* hybrid colonies.

Stage	*xyloni* type	Hybrid type
	Workers	
Larvae	0	23
Pupae	0	18
Adults	0	242
	Queens	
Larvae	8	0
Pupae	33	2
Virgin	100	4
Mated*	5	0
	Males	
Pupae	18	0
Adults	21	0

*Some of the samples analyzed here were progeny of these five nest queens.

suggest that the colonies studied were the F_1 generation of crosses between *S. xyloni* queens and *S. geminata* males (*13*). To test the hypothesis that the phenotypically *xyloni* type F_1 queens are of hybrid genotype, we attempted to find progeny of the F_2 generation in nature and to produce it by artificial insemination. A hybrid F_1 queen when crossed with a *xyloni* male should produce both hybrid and *xyloni* workers. Her male progeny should be both *xyloni* and *geminata*, reflecting the genotype of this F_1 queen. However, we examined 16 colonies and none of them had F_2 progeny. We also artificially inseminated 25 F_1 queens with *xyloni* males. In the artificial insemination of *S. invicta*, all queens undergo dealation after the insemination and lay eggs within 48 hours, whether the operation is successful or not (*14*). Yet these 25 F_1 queens could not break off their wings despite great effort, and all died within 3 days. Whether this failure in their dealation attempt prevents the F_1 queens from establishing their colonies and producing F_2 progeny needs to be further investigated.

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References and Notes

1. E. O. Wilson, *Bull. Mus. Comp. Zool. Harv. Univ.* **113**, 1 (1955); A. C. Cole, Jr., *Pogonomyrmex Harvester Ants* (Univ. of Tennessee Press, Knoxville, 1968), pp. 99–102.
2. M. W. Wing, *Cornell Univ. Agric. Exp. Stn. Mem. 405* (1968), p. 38.
3. E. W. Cupp, J. O'Neal, G. Kearney, G. Markin, *Ann. Entomol. Soc. Am.* **66**, 743 (1973). This technique has failed to work in our laboratory; there seem to be factors involved other than those described in their paper in the successful performance of this method.
4. A. C. F. Hung, unpublished results.
5. Using the technique developed for alcohol dehydrogenase of *Triticum* by G. E. Hart [*Mol. Gen. Genet.* **111**, 61 (1971)].
6. These larvae were subjected to electrophoresis shortly after the queen died. Queens of the re-

ciprocal crosses were not successfully inseminated.
7. C. L. Markert and H. Ursprung, *Developmental Genetics* (Prentice-Hall, Englewood Cliffs, N.J., 1971); R. P. Wagner and R. K. Selender, *Annu. Rey. Entomol.* **19**, 117 (1974).
8. J. B. Courtright, *Genetics* **57**, 25 (1967).
9. E. Castro-Sierra and S. Ohno, *Biochem Genet.* **1**, 323 (1968).
10. For a review of caste determination in ants, see E. O. Wilson, *Insect Societies* (Harvard Univ. Press, Cambridge, Mass., 1971), pp. 146–156.
11. The plastic periods of fire ant brood in caste determination have yet to be determined by more elaborate studies.
12. R. M. Robeau and S. B. Vinson. *J. Ga. Entomol-Soc.* **11**, 198 (1976).
13. Males that develop from unfertilized eggs reflect only the genotypes of their mothers. The diploid workers show the genotypes of both parents.
14. Fire ant queens will lay eggs even if not inseminated. However, these eggs are either inviable trophic eggs or unfertilized eggs that develop into males.
15. Approved as TA 13001 by the director of the Texas Agricultural Experiment Station. Supported in part by the Texas Department of Agriculture Interagency Agreement IAC (74–75)–0448. We thank R. H. Crozier, G. E. Hart, and P. Sroka for discussion; and W. L. Brown, W. F. Bruen, and E. O. Wilson for comments on the manuscript.

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Steroid Contraceptive Use and Cervical Dysplasia: Increased Risk of Progression

Abstract. *In a prospective study of women with dysplasia of the cervix, there was an increase in severity of dysplasia and of conversion to cancer in situ in users of the contraceptive pill compared with users of other contraceptive methods. There was a delay in this adverse response. Nonreversal of dysplasia within the first 6 months of pill use is predictive of progression after prolonged exposure.*

We investigated the possible association of steroid contraceptive use and carcinogenesis of the cervix by longitudinal observation of women with the cancer precursor, dysplasia of the cervix. All of the women in the study were using either the contraceptive pill or another method of contraception. The objective was to look for a differential effect of the pill on progression from dysplasia to cancer in situ. In this case, the possible carcinogenic effect of steroid contraception on dysplasia could be of a promoting rather than initiating nature.

According to the natural history model of stepwise progression in carcinogenesis of the cervix (*1*), dysplasia is an intermediate step from which progression to cancer or reversal toward normal may occur as in the following scheme:

normal ⇄ dysplasia → cancer in situ → invasive cancer → death

By limiting the scope of our study to progression from dysplasia to cancer in situ (*2*), we circumvented the problem of obtaining the large sample sizes that would be required in studying differential cancer incidence in normal young women.

Studies of the possible carcinogenic effect of the contraceptive pill on the cervix have yielded conflicting results (*3*). A greater prevalence of cancer in situ in women using oral contraceptives than in women using other contraceptive methods was reported (*4*), but an association of pill use with the development of cervical cancer was not substantiated after further follow-up by the same investigators (*5*). The higher prevalence may have been attributable to a self-selection factor, since women who chose the pill had a higher prevalence of dysplasia before ever using it than women who chose other contraceptive methods (*6*). Random allocation of a contraceptive method is unacceptable to women, and even though known differences between study and control groups have been taken into account in our analyses, the problem of self-selection in a nonrandomized study is never completely resolved.

Our study design enabled us to monitor other possible confounding factors such as changes to other methods or cessation of contraception, or subjects becoming pregnant or dropping out of the study. Confounding effects resulting from pregnancy or changes in method of contraception were avoided by excluding the data on such women from the time such an event occurred. Dropout rates or reasons for dropping out did not differ according to the contraceptive method used. The question of possible bias due to differences in the characteristics of those subjects who continued in the study and those who dropped out was an important consideration, and our analysis of subjects by dropout status and contraceptive method revealed no evidence that dropping out of the study was related to disease outcome (*7*). Another important source of artifact specific to studies of cervical neoplasia and pill use is the Papanicolaou (Pap) test that is given preferentially to users. We avoided this problem by baseline assessment of the cervical mucosa of all subjects before the steroid contraceptive was ever used, and by equal application of test procedures to pill users and nonusers over the duration of the study.

The subjects were selected from approximately 11,000 women enrolling sequentially in Los Angeles County family planning clinics from 1967 through 1971, the cutoff for entry. Even at their first visit almost 50 percent of the women had already used the contraceptive pill and were thus ineligible for the study; others were excluded because of a history of illness that could bias the free choice of contraceptive method. From almost 6000 eligible women (*8*), Pap screening identified 300 women with dysplasia. A sample of 300 women (5 percent) with negative smears was selected concurrently and followed in the same way as the women with dysplasia. Subjects were examined again at 2 months, 6 months, and then every 6 months. The status of the cervical mucosa was monitored by Pap smear, and this information was supplemented in the women with dysplasia by annual biopsy limited to superficial samples of the mucosa (*9*). All cytologic and histologic evaluations were performed blind. Women using the steroid contraceptive pill (users) were compared to women using all other contraceptive methods combined (nonusers) (*10*). Over 90 percent of the pill nonusers used an intrauterine device (IUD). The pill was limited to one progestin-estrogen compound at one dose level throughout the study (*10*). Both study and control women used contraceptives and were thus presumably sexually active.

In this study of carcinogenesis in young women, average age 23 years, using contraception, we found that conversion to cancer in situ was slow even in women who already had dysplasia and were thus at high risk. We therefore also examined transitions over the range of dysplasia, assuming that conversion to cancer involves an ordered progression through increasing degrees of severity of dysplasia (*11, 12*). As an aid to measuring degrees of progression, we constructed a numerical scale to cover the entire gradient of abnormality in the cervical mucosa (*12*). It was then possible to relate oral contraceptive exposure to change in diagnostic score over time (*13*) (see Fig. 1).

In the sample of normal women there is as yet no evidence of a differential effect of the pill (Fig. 1A). However, in the dysplasia sample, there was a differential effect over time, and after 2 years the pill users maintained higher scores than the nonusers for the duration of the study. The differential effect is enhanced after scores are adjusted for concomitant variables (*7*). The decline in scores after 2 and 6 months is also of interest. The early drop is greater, though not significantly so, in the pill users than in the