the slide with a Pasteur pipette. A second quartz slide was placed on top of the first, and the eggs were flattened between the two slides by exerting firm but gentle pressure on the ends of the upper slide over the permoplast.

The slides were placed at a distance of 18 inches (1 inch = 2.54 cm) directly over an ultraviolet lamp (Mineralite model R-51). This particular model delivers 155 μw/cm^2 (or 930 erg min^{-1} mm^{-2}) at a distance of 18 inches. Germ cell formation was completely suppressed when the vegetal hemispheres were exposed to the irradiation for 45 minutes. Parabiosis in the future gill region was accomplished at the tailbud stage of development (stage 17 of Shumway, *10*) by joining unirradiated embryos with either irradiated (experimental) embryos or other unirradiated (control) embryos. At Shumway stage 25, the parabionts were anesthetized in MS-222 (1:10,000 concentration of tricaine methanesulfonate; Sandoz Pharmaceuticals, Hanover, N.J.), carefully eviscerated, and photographed.

The results of eight replicate experiments were uniformly consistent. Unirradiated pairs of embryos examined at Shumway stage 25 invariably displayed normal gonad development. Each embryo possessed two conspicuous genital ridges containing large primordial germ cells (Fig. 1A). In marked contrast to unirradiated pairs of embryos, no primordial germ cells were seen in irradiated embryos joined to normal partners possessing the usual two rows of primordial germ cells (Fig. 1B). Only sterile genital ridges were present in the irradiated embryos.

The observed absence of primordial germ cells in irradiated parabionts is interpreted as indicating that the circulatory system does not transport germ cells in the embryos of *Rana pipiens*. The possibility exists that a few germ cells might gain entry into the circulating blood but remain undetected because of their failure to reach the definitive site in the genital ridges. This seems unlikely, however, particularly if the successful colonization of primordial blood cells in hematopoietic organs in parabiotic frogs is taken as a dramatized demonstration of the strong homing tendency of cells (*15*).

Negative data are rarely satisfying, but we may conclude by stating that our results do not lend support to a thesis that would advocate the migration of amphibian germ cells through blood vessels. The explanation that now seems most reasonable is Humphrey's contention, dating back to 1925 (*8*), that the primordial germ cells of amphibians reach their final position in the genital ridge entirely by mechanical displacements accompanying embryonic growth.

E. PETER VOLPE
SHERILL CURTIS
Department of Biology, Tulane University, New Orleans, Louisiana

References and Notes

1. D. Simon, *Compt. Rend. Soc. Biol.* **151**, 1010 (1957); *Arch. Anat. Microscop. Morphol. Exp.* **49**, 93 (1960).
2. D. B. Meyer, *Develop. Biol.* **10**, 154 (1964).
3. R. P. Singh and D. B. Meyer, *Science* **156**, 1503 (1967).
4. E. Witschi, *Contrib. Embryol. Carnegie Inst. Washington* **32**, 67 (1948); B. Mintz and E. S. Russell, *J. Exp. Zool.* **134**, 207 (1957).
5. S. Ohno, J. M. Trujillo, C. Stenius, L. C. Christian, R. L. Teplitz, *Cytogenetics* **1**, 258 (1962).
6. S. Ohno and A. Gropp, *ibid.* **4**, 251 (1965).
7. K. Benirschke and L. E. Brownhill, *ibid.* **2**, 331 (1963).
8. R. R. Humphrey, *J. Morphol. Physiol.* **41**, 1 (1925).
9. B. M. Allen, *Anat. Anz.* **31**, 339 (1907).
10. Embryonic stages defined and illustrated in W. Shumway, *Anat. Rec.* **78**, 139 (1940).
11. E. P. Volpe and B. M. Gebhardt, *Science* **154**, 1197 (1966).
12. L. D. Smith, *Develop. Biol.* **14**, 330 (1966).
13. L. Bounoure, R. Aubry, M. L. Huck, *J. Embryol. Exp. Morphol.* **2**, 245 (1954); E. Padoa, *Estratto Monit. Zool. Ital.* **70**, 238 (1963).
14. R. Rugh, *Biol. Bull.* **66**, 22 (1934).
15. E. P. Volpe and B. M. Gebhardt, *Exp. Cell Res.* **49**, 194 (1968).
16. Supported, in part, by a PHS biomedical sciences research grant to Tulane University and in part by PHS grant GM-11782.

29 January 1968

Chick's Response to an Imprinting Stimulus: Heterosis and Evolution

Abstract. *Tendencies of day-old chicks to respond toward, to approach, and to stay near a distant audiovisual imprinting apparatus were shown to be inherited in a heterotic manner. High correlations found among the traits suggest that these behavioral responses are not independent but sequential. The fitness value of such a behavioral sequence is explained in terms of imprinting.*

The formation of initial social bonds by precocial neonates is a form of behavior imprinting which, although widely investigated, remains controversial; the phenomenon consists of a complex of events. Thus investigators must either go to great lengths to show that the trait quantified is really related to imprinting, or (more often) speak of such factors as the "approach response" and "the following response," which are assumed to be related to imprinting. Although approach and following may be involved, maintenance of the following response may not necessarily reflect a preference for the object followed (*1*), and response and approach to an imprinting apparatus have not been shown to be a measure of a chick's tendency to imprint (*2*).

Our purpose was to determine whether response toward, approach to, and tendency to stay near a parent surrogate during the critical period for imprinting are adaptive traits, and to determine the relations among these traits. Demonstration of heterotic inheritance of the traits measured would greatly strengthen the argument that they are components of imprinting.

The subjects were 669 domestic chicks from five 2 × 2 mating sets (*3*). Since one of the lines was used in two mating sets, there were nine types of F_1 purebreds (290 chicks) and ten types of F_1 crossbreds (379 chicks). Eggs were collected daily and stored at 13°C until incubation; after 18-day incubation they were placed in individual compartments in a dimly lighted hatcher (maximum light intensity, 4.3 lux). Compartments were inspected at 2-hour intervals, and when a chick had hatched (completely emerged from the egg) it was transferred to a labeled compartment until testing.

The testing compartment, developed from one described (*4*), was hexagonal and measured 90 cm between parallel sides. The visual stimulus consisted of a translucent plastic dome containing moving colored lights that flickered on

Table 1. Weighted phenotypic correlations among components of the approach response; *N*, 535.

	Response, no response	Time to approach	Approach, no approach	Time near apparatus
Time to respond	.81	.75	.80	—.81
Response, no response		.67	.70	—.54
Time to approach			.96	—.83
Approach, no approach				—.78

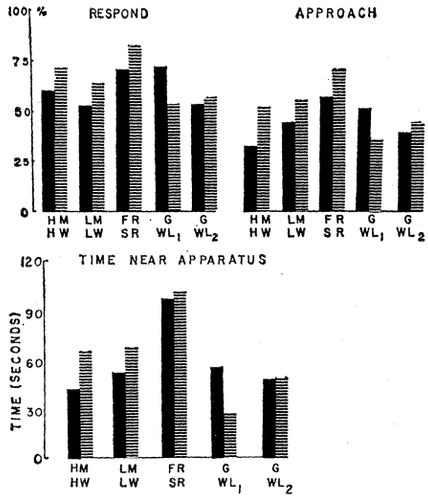

Fig. 1. Average response to, approach to, and time spent near the imprinting apparatus by purebreds (solid bars) and crossbreds (striped bars); *N*, 669.

and off. A continuous tape recording of the clucking of a broody hen furnished an auditory stimulus. The temperature in the testing room was maintained at about 30°C. Each chick was given one 5-minute test.

Each chick was transported from its isolation box to the testing compartment 24 ± 2 hours after hatching and placed in a circle, 5 cm in diameter, drawn on the floor of the compartment opposite the visual stimulus. Time before response toward the stimulus, time before approach to the stimulus (within 15 cm), and time spent near the apparatus (within 15 cm) during the test period were the behavior traits measured.

A trend is obvious in response, approach, and stay-near tendencies of purebred and crossbred chicks (Fig. 1). In four of the five comparisons crossbreds were superior to purebreds in response and approach tendencies. Chi-squares (variances were heterogeneous) comparing all crossbreds with all purebreds showed that significantly more crossbreds than purebreds responded and approached. Differences within mating sets were not significant, but this fact is not surprising since variances for these traits are large. Essentially the same pattern is seen for the tendency of chicks, 24 hours after hatching, to stay near the imprinting apparatus; variances were homogeneous for this trait but still very large. Results of *F* tests comparing crossbreds and purebreds within 2 × 2 sets were not significant, but pooling of data over sets showed that crossbreds spent significantly more time near the apparatus than did purebreds.

Although the degree of inbreeding of the lines employed was low in most instances (*3*), results showed that tendencies to respond toward, to approach, and to stay near a distant imprinting apparatus 24 hours after hatching are heterotically inherited traits. The results do not preclude additive inheritance, which has a low but significant effect on response tendencies (*5*).

Fisher's fundamental theorem of natural selection (*6*) predicts that traits that are major components of fitness contain little additive genetic variation (σ_A^2) when the population concerned is in relative equilibrium with its environment. Such traits will have been subjected to extensive prior selection that reduces σ_A^2 but favors the accumulation of (or evolution of) nonadditive genetic variation. According to this theory the traits measured by us were probably adaptive during development of the domestic fowl.

Correlations between selected and unselected traits may change during the course of selection, and genetic correlations may in fact change sign during a selection experiment (*7*). For this reason the FR and SR (*3*) and their reciprocal crosses were excluded from the correlation analysis.

Table 1 shows weighted phenotypic correlations among the traits studied; both quantitative and qualitative measurements of response and approach tendencies are shown. The fact that response toward the imprinting apparatus correlated highly with approach and with time spent near the apparatus indicates that chicks that respond generally follow a pattern of approach and stay-near behavior. This sequence of steps seems to be meaningless except in terms of the concept of imprinting. The evidence that nonadditive gene action has evolved for response, approach, and stay-near behavior during the critical period for imprinting in this neonatal species, coupled with evidence that such behaviors are highly related, supports the idea that these traits indicate tendencies to imprint.

H. B. Graves
P. B. Siegel
Department of Poultry Science, Virginia Polytechnic Institute, Blacksburg 24061

References and Notes

1. P. H. Klopfer, *Behav. Sci.* **12**, 122 (1967).
2. H. B. Graves and P. B. Siegel, *Anim. Behav.* **16**, 18 (1968).
3. The abbreviations used and inbreeding coefficients (*F*) for the lines are HM and LM, high and low mating lines (*F*, .16); HW and LW, high and low body weight (*F*, .16); FR and SR, fast and slow response (*F*, .10); WL1 and WL2, white leghorn lines (*F*, .54 and .27, respectively); G, game (*F*, unknown).
4. F. V. Smith and M. W. Bird, *Anim. Behav.* **11**, 300 (1963).
5. H. B. Graves and P. B. Siegel, *Bull. Ecol. Soc. Amer.* **47**, 200 (1966).
6. R. A. Fisher, in *The Genetical Theory of Natural Selection* (Clarendon Press, Oxford; Dover, New York, 1958), p. 37.
7. J. M. Rendel, *Genetics* **48**, 391 (1963).

1 March 1968

Reinforcement Magnitude as a Determinant of Performance Decrement after Electroconvulsive Shock

Abstract. *The intensity of a foot shock may be a determinant of the rate at which an avoidance response becomes resistant to disruption by electroconvulsive shock. Mice were trained, one trial a day, in a passive avoidance learning task, with one of three foot-shock intensities. Electroconvulsive shock was administered at various intervals after each trial. At all foot-shock intensities, electroconvulsive shock given 10 seconds after each training trial was effective in disrupting learning. Where electroconvulsive shock was given at longer intervals after each trial, those animals learning at low intensities of foot shock showed greater impairment of performance than those learning at high intensities.*

The relationship between retention of a learned behavior and the administration of electroconvulsive shock (ECS) has been studied extensively. Primary emphasis has been on the interval between training and ECS delivery. Typically, the longer this interval, the less performance is disrupted when the animals are later tested. There is debate, however, over the duration of the period after learning during which ECS disrupts memory consolidation (that is, causes retrograde amnesia). Chorover and Schiller (*1*) place an upper limit of 10 seconds on the interval during which ECS can produce retrograde amnesia. Quartermain *et al.* (*2*) have suggested a 30-second limit; Mc-