Inheritance of Two Alkaline Phosphatase Variants in Fowl Plasma

Abstract. *Starch-gel electrophoresis of plasma reveals two mutually exclusive forms of alkaline phosphatase. Analysis of the plasma of 931 birds from 96 sib families of two inbred lines through two generations shows that the faster-moving isozyme is determined by an autosomal gene* Ap^2 *which is completely dominant to its allele* Ap^4 *determining the slower form.*

Electrophoresis of alkaline phosphatase isozymes, from many sources, on starch gels indicates they are under genetic control. Specific reports on *Drosophila melanogaster* (*1*), sheep (*2*), cattle (*3*), and humans (*4*) have been made in this regard. Quantitative studies have shown (*5*) that the amount of alkaline phosphatase in the serum of the domestic fowl is under genetic control, but to our knowledge there are no prior reports of this enzyme being shown by electrophoresis to have more than one form.

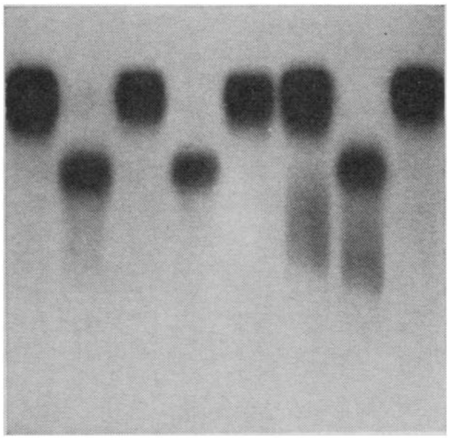

Fig. 1. Starch gel electrophoresis of chicken plasma showing two zones of alkaline phosphatase activity. (0.04*M* tris buffer adjusted to *p*H 8.6 with boric acid, 5 volt cm^{-1} for 16 hrs, stained with fast blue RR as a dye coupler and α-naphthyl phosphate as substrate. Origin at bottom, direction of migration upwards.)

Table 1. Frequency of progeny phenotypes from various matings in two inbred lines. (Under Matings, 22 = Ap^2/ Ap^2; 24 = Ap^2/ Ap^4; 44 = Ap^4/Ap^4. Parent genotypes were determined by a combination of phenotype and progeny test or parentage, except ? = unknown.)

Matings (♂♂ × ♀♀)	Progeny phenotypes	
	Ap^2 (fast)	Ap^4 (slow)
	Inbred line 45	
22 × 24	67	0
24 × 22	17	0
24 × 24	81	19
24 × 44	15	15
44 × 24	25	24
44 × 44	0	84
	Inbred line 79	
22 × 2?	71	0
22 × 24	114	0
22 × 44	61	0
24 × 2?	9	0
24 × 24	54	15
24 × 44	19	26
44 × 22	66	0
44 × 44	0	149

Heparinized blood samples were obtained from birds 3 to 4 weeks of age, at which time the amount of alkaline phosphatase is relatively large. Electrophoresis was conducted in horizontal gel trays (*6*). The staining was a dye-coupling procedure (*7*). Gels were preserved in a mixture of methanol, water, and acetic acid (50:50:10) until photographed by reflected light on high-contrast copy film. This procedure provided zymograms which were clearly differentiated and easily interpreted. As yet we have seen only two forms of the enzyme, one of which moves decidedly faster than the other (Fig. 1).

We have examined plasma samples from more than 900 birds of two individually pedigreed lines and surveyed an additional 2000 samples from eleven other lines. We have never found more than one isozyme in any one plasma. Analysis of the pedigreed families showed that the fast-moving form is determined by a simple autosomal dominant gene which is allelic to a recessive gene responsible for the slower isozyme. We have assigned the symbols Ap^2 and Ap^4 to the genes for fast and slow components, respectively. In a heterozygous individual Ap^2 excludes the appearance of the product of the Ap^4 allele. Thus, birds having the slow form are homozygous recessive (Ap^4Ap^4). Birds possessing the fast form may be homozygous dominant (Ap^2Ap^2) or heterozygous (Ap^2 Ap^4).

The evidence for the theory of genetic control by two alleles with complete dominance is based on data collected on two generations in two inbred lines. We studied a total of 16 sires, 96 dams, and 931 offspring. The data are summarized in Table 1. Since the classes of progeny fit the 1:0, 1:1, 3:1, or 0:1 ratios expected with simple dominance ($\chi^2 = 0.13$) and there are no differences between reciprocal matings, an autosomal locus controls the two phenotypes. Why the Ap^2 gene in the heterozygous chicken should exclude the product of its Ap^4 allele from appearing in, or being detected in, the plasma is unknown.

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Mirror-Image Reversal in Pigeons

Both Levine (*1*) and Mello *et al.* (*2*) have noted that light passes readily from one eye of the pigeon to the other through the paper-thin interorbital septum. In the latter case this trans-septal transmission of light occurred even though the pigeons were wearing goggles which somewhat restrict the peripheral field of view. One of us (Siegel) has observed that if one of the pigeon's eyes is enucleated and a bright stimulus presented to the remaining eye, the focused image of the stimulus appears on the septum at the rear of the evacuated orbital cavity. Since the retina normally abuts the septum at this point in the intact pigeon, this observation implies that, under some circumstances, a stimulus presented to one eye of the pigeon could stimulate the contralateral eye through this rear projection system. An image propagated trans-septally would not only be greatly attenuated in intensity, but would be the mirror image of what it would have been had it traveled the more usual route to the retina.

Interpretation of Mello's report (*3*), of maximal generalization with the "untrained eye" to the mirror image of an oblique line used in training the other eye, is complicated by this possibility, particularly since the sensitivity of the covered eye was raised by dark adaptation during training. The reversal may be *interocular* rather than "interhemispheric," and the pigeon may be less unique neuroanatomically than Mello implies. Since she does not present details of the goggles used in the experiment, their ability to constrict the visual field to an area away from the interorbital septum cannot be evaluated. A possible control for trans-septal propagation of the image would be to reduce the general level of sensitivity of the "untrained eye" by maintaining it in a state of light adaptation throughout the training phase and thus preventing reception of the relatively weak stimuli projected by the "trained eye."

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Monocularly trained pigeons, previously reinforced for pecking at a 45° oblique line (╱), respond maximally to its mirror image, 135° (╲), during generalization tests with the opposite, untrained eye open (*1*). Cumming, Siegel and Johnson have speculated that a pattern falling upon the retina of the "trained" eye may simultaneously stimulate the retina of the opposite "untrained" eye via the bony septum in the mid-sagittal plane, thereby providing a mirror image of the training stimulus. At least three lines of evidence argue against Cumming, Siegel, and Johnson's suggestion.

1) Examination of the gross anatomy of the pigeon shows that only the anteromedial portions of the orbits lie back to back (Fig. 1). The anteromedial portion of the retina subserves the lateral visual field of the pigeon, which was occluded by the goggles in the behavioral experiments (*1, 2*). The discriminative stimuli could not fall upon the anteromedial retina, regardless of the orientation of the head. Since the pigeons pecked at discriminative stimuli which were projected upon the response key, the mirror-image patterns fell within the frontal visual field, which stimulates the temporal rather than the anteromedial portion of the retina. The temporal retina comprises the posterolateral portion of the orbit, which is adjacent to the brain. This anatomical arrangement precludes the possibility that an image projected on the temporal retina of one eye could traverse the intervening tissue and reach the retina of the opposite eye.

2) This anatomical point has also been demonstrated electrophysiologically (Fig. 2).

As Cumming, Siegel, and Johnson have noted, our earlier experiments did show that if a stroboscopic flash to one eye stimulated both retinas by transmission of light across the septum, evoked potentials of comparable form and latency were obtained from *both* optic tecta (*2*). Cumming *et al.* incorrectly infer that the "peripheral field of view" was restricted in these experiments. In fact, these bilateral evoked potentials occur simultaneously *only* if the photic stimulus falls within the pigeon's lateral visual field, where the orbits are in direct apposition (Fig. 2; *3*). If a stroboscopic flash is confined to the frontal field of one eye, it does not simultaneously evoke a potential from the ipsilateral optic tectum (*2*; Fig. 2). The data shown in Fig. 2 were obtained from a pigeon in which one optic tectum had been totally ablated. This preparation was necessary since flash confined to one eye evokes a potential from the contralateral optic tectum (*1*; see Fig. 2) and a secondary, late potential from the ipsilateral optic tectum (*2*).

These data argue against the notion that the relatively dim stimuli (about 2.5 meter-lamberts) used in the behavioral experiments (*1*) could have affected the "untrained" eye.

We have attempted to extend Siegel's observation by presenting discriminative stimuli used in the behavioral experiments to the lateral visual field of an anesthetized pigeon in which the vitreous of the opposite eye had been evacuated, leaving the pigmented epithelium and sclera intact. Although the image was directed towards the only portion of the orbit that could transmit light to the opposite eye, only a diffuse light was detectable.

Table 1. Responses during extinction tests, obtained from pigeons monocularly trained to discriminate left-right, mirror-image T's. S+ is the stimulus associated with reinforcement for responding during training and S− is the stimulus never associated with reinforcement.

Pigeon	Open eye			
	Trained		Untrained	
	S+	S−	S+	S−
A	692	130	68	293
B	712	5	79	140
C	27	0	4	63

3) Pigeons also show interhemispheric reversal of a left-right mirror-image pattern discrimination even when the "untrained" eye remains light adapted throughout training. Cumming *et al.* have suggested light adaption of the "untrained" eye during discrimination training as a test of their hypothesis.

As part of a separate experiment, three pigeons were monocularly trained to discriminate left-right, mirror-image T's (⊢, ⊣) according to the procedure described in the second experiment of the previous report (*1*). The "untrained" eye was covered with a translucent diffusing filter. Diffuse light was provided both by the discriminative stimuli and by the light in the food magazine. Table 1 shows that

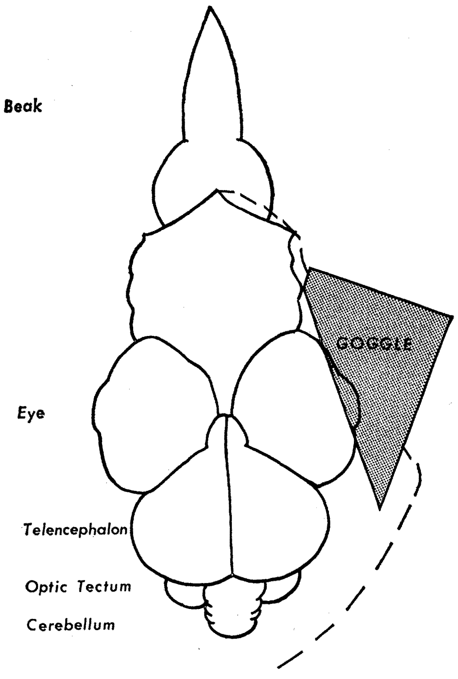

Fig. 1. Gross anatomy of the pigeon head. This diagram was made by tracing a projected photograph of a dissection of a pigeon head. This tracing shows the relative position of the eyes and beak with respect to the brain. A goggle like those used in the behavioral experiments is shown in its usual position over the right eye. The dotted line represents the margin of the dental acrilic used to attach the goggle to the scalp.

even under these conditions each pigeon responded to the stimulus trained as negative (S−) as if it were positive during tests of interocular transfer with the untrained eye alone open.

Thus evidence from anatomical, physiological, and behavioral sources argues against Cumming, Siegel, and Johnson's proposal that interhemispheric transfer of a monocularly learned pattern discrimination is literally "inter-ocular."

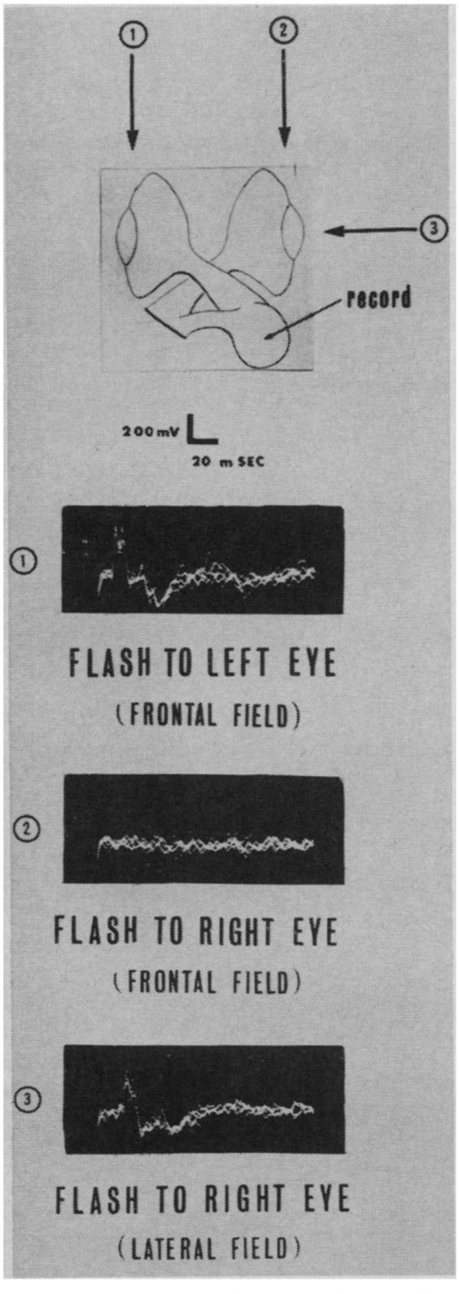

Fig. 2. Evoked potentials to flash recorded from the right optic tectum of the pigeon with a chronic ablation of the left optic tectum. Small, tungsten in glass electrodes (about 10 mμ tip) were used. The pigeon was anesthetized with nembutal (30 mg/kg). Flash stimuli were presented at 1 per second at maximum intensity through a black cone directed towards the orbit. The terminal aperture was 1.6 cm in diameter.

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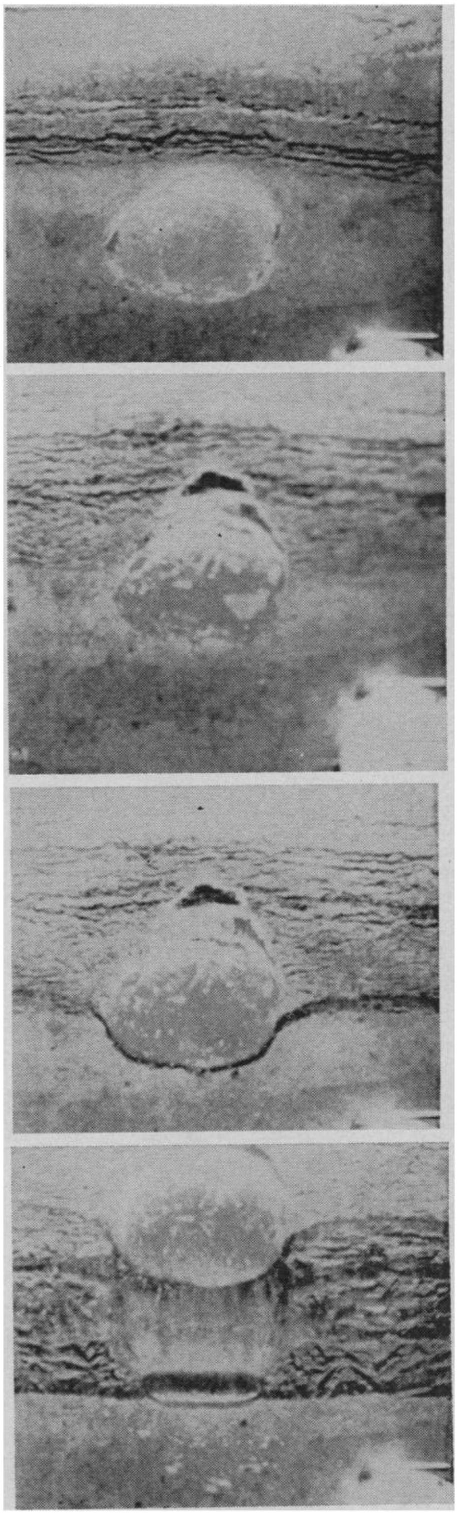
Stone Migration by Freezing of Soil

I have read with great interest D. R. Inglis's explanation of the uplifting of a large object such as a stone or fence post during freezing of soil [*Science* **148**, 1616 (1965)]. As early as 1958, using time-lapse color photography, I made observations of this phenomenon at the U.S. Army Arctic Construction and Frost Effects Laboratory, Waltham, Massachusetts (now merged with U.S. Army Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire). A 15-minute sound and color film entitled "Frost action in soils" was presented at the International Permafrost Conference at Purdue University in November 1963, and has been in continued circulation since. It has been shown at various gatherings of the American Society of Civil Engineers. It has also been shown in Japan and Sweden.

This film demonstrates, among other things, the upward movement of a stone and simulated piles within a silt soil by frost action, when freezing is from the top down in an open-system test. The narration accompanying the film is in very close agreement (with some exceptions) with the explanation set forth by Inglis. The film shows that adhesion of ice to the top of the stone was not the lifting force, although it might possibly be under certain conditions of object shape and relative position. What actually happened in the frost-susceptible material studied was that the soil directly over a stone was lifted above the stone, leaving a void. A stone rises only when the adfreeze force around it is greater than the forces holding it in place. The total movement of the stone from its initial position depends upon the heaving rate of the soil and the time required for freezing to penetrate down to a level below the cavity formed under the stone. In saturated non-frost-



Figs. 1–4 (top to bottom). Movement of a stone (actual size, about 3 cm diameter) in freezing soil. (The object partly visible at the lower right is a wrist watch.) Fig. 1: frost line approaching the top of the stone. Fig. 2 (about 30 hours later): the soil above the stone has heaved, leaving a void. Fig. 3 (about 12 hours later): the stone is being lifted by the grip of the frozen soil; there is now a void under the stone. Fig. 4 (about 79 hours later): the stone has moved up a considerable distance, and the cavity below has become slightly narrower and filled with water, which has frozen.