of anxiety during the procedure and they had V-A differences of 1.2 and 0.4 ng/ml. The latter value is toward the lower end of the range (see Table 1) and is not consistent with a simple relationship between anxiety and the magnitude of the V-A difference.

Concerning the degree to which urinary MHPG reflects brain NE metabolism, the following points should be noted. In addition to MHPG, dihydroxyphenethyleneglycol (DHPG) is an important metabolite of brain NE (4, 14), and it is likely that a large fraction of this product on entering the body pool is O-methylated to form MHPG. Thus, the portion of the MHPG that is derived from DHPG, and therefore from brain NE metabolism, is not accounted for in the estimates given here. On the other hand, some of the MHPG formed in brain, on entering the body pool, may be converted to other products such as 4-hydroxy-3-methoxymandelic acid ["vanillylmandelic acid" (VMA)] and thus may not appear in urine as MHPG. For example, one group of investigators found that after an injection of deuterated MHPG into monkeys, 7 percent of the label was recovered as VMA; in contrast, another group found no labeled VMA in urine during a 24-hour period after the infusion of [³H]MHPG into the internal jugular bulb of the monkey; and a third report indicates that in an infant with a neuroblastoma, 27 percent of an injected bolus of [³H]MHPG was recovered as [³H]VMA (15). Also, the mean $P_a CO_2$ for the subjects was 33.9 ± 1.1 torr, which is below the normal value of 40 torr, and because hypocapnia decreases CBF the actual CBF may have been less than that used to compute MHPG production per brain per minute.

In future work with humans, measurement of the V-A difference for a neurotransmitter metabolite coupled with a direct measure of CBF should yield information on brain neurotransmitter systems that will be of importance to investigators from a variety of disciplines.

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Pigeons Have Magnets

Abstract. *Research on pigeon homing suggests that magnetic field information is used for orientation. The ability of pigeons to sense magnetic fields may be associated with a small, unilateral structure between the brain and the skull which contains magnetite in what appears to be single domains.*

Many homing pigeons are able to find their way home after being released at an unfamiliar site. They typically take up an appropriate homeward flight direction shortly after release. To do this, the birds must know where they are with respect to home and be able to judge direction; that is, they must have both a "map" and a "compass sense" (1). On sunny days, their compass appears to depend on the sun since clock-shift experiments predictably deflect departure bearings (2). Since the same clock-shifted pigeons depart directly toward home on cloudy days, some nonsolar backup compass must be available (3). Because the ability of pigeons to orient on cloudy days is disrupted when small magnets (4) or paired coils (5) generating a magnetic field are affixed to their heads, it seems likely that the backup compass is partly or wholly magnetic, and is located in the head or neck. Since the initial homeward orientation of pigeons on sunny days is slightly affected by paired coils (6) and magnetic storms (7), and can be virtually abolished when the birds are released at strong magnetic anomalies (8), it is possible that the sunny-day compass has a magnetic com-

ponent or that the mysterious "map" system may utilize magnetic field information.

Very little is known about how pigeons might sense the earth's field. However, we know of only three general strategies which an organism might use to detect magnetic direction. One method would be to measure the electric field (that is, charge separation) generated by moving a conductor through a magnetic field. This is almost certainly the system employed by elasmobranch fishes (9). Since the weak, static field of a set of coils attached to (and hence moving with) a pigeon nevertheless disrupts cloudy-day homing (5), the compass probably does not involve an induction detector. A second possibility would be to use permanent magnets, perhaps by measuring the torque generated as they attempt to twist into alignment with the earth's field (10). Chitons (11), honey bees (12), and many species of mud bacteria (13) do possess single domains (tiny unit magnets) of magnetite which, excepting chitons, may be used for magnetic field orientation. The third strategy might be to sense the effects of paramagnetic fields which are produced by

many substances as a result of a temporary alignment of the spins of their unpaired electrons with an external field. Many organic molecules are weakly paramagnetic and, in addition, honey bees possess large numbers of superparamagnetic crystals of magnetite (12) that are too small to have stable, permanent fields of their own at room temperature, and so behave like paramagnetic substances. In an earth's field, superparamagnetic crystals would align their own fields with those of the earth. Depending on the crystal spacing and relative orientation, these superparamagnetic domains would attract or repel each other in a way which could specify the direction of the earth's field (10, 13, 14).

We tested for stable and superparamagnetic domains in pigeons with a SQUID magnetometer (15). Since the paired coil experiments indicate that the compass is in the head or neck (5), we dissected approximately two dozen fresh, previously frozen, or perfused pigeon heads and necks with nonmagnetic tools and looked for any natural remanence inducible at room temperature with either a 700- or 2000-gauss magnet (a test for stable permanent magnetism), and any inducible remanence when the head and neck sections were chilled to $-196°C$ in liquid nitrogen (a test for superparamagnetic domains in the 200- to 400-Å size range) (11). When remanence was found, the sample was progressively subdivided until the site of magnetic activity was located.

In each of the pigeons tested we found naturally (that is, permanently) magnetic material. The material was always unilateral and was located either in a small (1 by 2 mm) piece of tissue between the dura and the skull or was too closely associated with the skull to be separated from it conveniently. About 40 percent of the pigeons had a natural remanence (a net field due to a preferential direction of alignment among the many individual magnets) which ranged from 10^{-7} to 10^{-6} electromagnetic unit, while all had an inducible remanence (a measure of the total amount of magnetic material) of 10^{-6} to 10^{-5} electromagnetic unit. The relatively weaker natural remanence and other observations suggest that the alignment among the single-domain magnets is only locally regular.

We froze the naturally magnetic tissues in liquid nitrogen and induced a remanence with a 3000-gauss magnet and then watched the remanence decay as the sample warmed up to room temperature. We observed no abrupt decrease

in remanence indicating that the tissue contains few, if any, superparamagnetic domains. Honey bees, on the other hand, possess a very large superparamagnetic component (12). We examined the rest of the pigeon head and neck for superparamagnetic domains and found small amounts of remanence only in the half of the head opposite the permanently magnetic material.

We have also examined the tissue in a very preliminary way with both the light and electron microscope. The tissue contains nerve fibers (16) and connective tissue and is richly supplied with clusters of electron-opaque structures approximately 0.08 to 0.15 μm long and with approximately a length-to-width ratio of 4:1, dimensions which would yield stable single domains if composed of magnetite (17) or maghemite, but superparamagnetic domains if they were crystals of any of a number of other naturally magnetic substances such as hematite (18). If the material is magnetite, this crystal size and the magnitude of the inducible remanence means that approximately 10^7 to 10^8 single-domain magnets are present. An electron probe analysis revealed that these particles are rich in iron, the primary component of most magnetic materials. Small but measurable amounts of nickel, copper, zinc, and lead were also detected. Similar trace elements have been found in magnetite crystals that are synthesized by chitons (19). X-ray analysis confirmed the presence of iron (20).

Given the strength of the remanence and the absence of other elements, the magnetic material must be either magnetite or maghemite (or both). To distinguish between these two minerals, we removed and pooled the magnetic tissues from five pigeons to obtain a Curie temperature. The remanence of maghemite disappears between 300° to 400°C as maghemite inverts to the weakly magnetic material hematite. The remanence of hematite in turn is lost at 680°C. The remanence of magnetite, on the other hand, disappears at about 580°C. We measured a Curie point for the pigeon tissue of 575° ± 10°C. In addition, we examined the crystals under the light microscope. Maghemite crystals are orange while of all magnetic minerals only magnetite is black. This is because the octahedral exchange coupling in magnetite absorbs photons of almost any energy level. Although the crystals in the pigeon tissue are too small to produce a color individually, the aggregations can and are unambiguously black. We conclude, therefore, that the primary magnetic component is magnetite.

The tissues also contain an easily shattered yellow crystal about 6 μm in diameter. Similar crystals can be formed by the iron storage protein ferritin, which may be involved in the synthesis of magnetite (21). Such a crystal would have no remanence and would contain sufficient iron to synthesize approximately 2×10^5 domains of magnetite. Electron diffraction analysis should establish this crystal's identity conclusively. Given its color, the total measured remanence, and the Curie temperature measurements, however, we see no way that this yellow crystal could by itself account for more than a tiny fraction of the magnetic properties of this tissue.

These results do not prove that pigeons actually use the innervated, magnetite-rich, naturally magnetic structure reported here as a magnetic field detector. Only behavioral and physiological experiments can actually determine whether or not pigeons use this presumptive detector in either their "map" or compass systems, and if so, how.

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Repeated Copulation and Sperm Precedence: Paternity Assurance for a Male Brooding Water Bug

Abstract. *Male giant water bugs* (Abedus herberti *Hidalgo) brood eggs attached to their backs by their mates. Brooders risk being "cuckolded" because females store sperm from previous matings. Males always copulate with females prior to receiving their eggs and mate repeatedly during oviposition. Experiments with a genetic marker reveal almost complete sperm precedence for the last male to mate with a female. The male's behavior therefore assures his paternity of the eggs he broods.*

A male should only care for, and take risk in defense of, young that possess his genes. Failure to assure paternity prior to investing in young may put a male at a selective disadvantage in competition with more reproductively selfish individuals (*1*). Observational and experimental studies on birds imply support for this thesis; however, few quantitative data are available to evaluate the efficacy of presumptive "anticuckoldry" adaptations (*2*).

With a few exceptions (*3*), male insects contribute nothing to reproduction beyond sperm. Among the exceptions are males of the giant water bug subfamily Belostomatinae, which invest time and energy brooding eggs attached to their backs by conspecific females (*4, 5*). *Abedus herberti* Hidalgo males brood by aerating eggs and assisting nymphs during eclosion (*5*). In addition, encumbered males do not feed while nymphs are hatching from their backs (*5*). This paternal behavior involves added risks, reduces predatory efficiency, and precludes additional mating for brooding males (*5, 6*). Male brooding, however, is essential for embryonic development under natural conditions (*4, 5*).

Ridley points out that the evolution of exclusive male nurture is correlated with external fertilization, presumably for reasons of paternity assurance (*3*). This is not the case for giant water bugs. Most female insects, including giant water bugs, store sperm in organs (sperma-

thecae) and use it to fertilize eggs laid over an extended period. *Abedus herberti* is long-lived (> 1 year), and an individual female of this species may mate with several males (theoretically as many as 12) in its lifetime (*6*). Thus, it is entirely possible for males to receive and brood eggs from previously mated females. Given this risk, selection should have favored mechanisms that would assure the brooding male's genetic contribution to eggs he carries. Indeed, a high
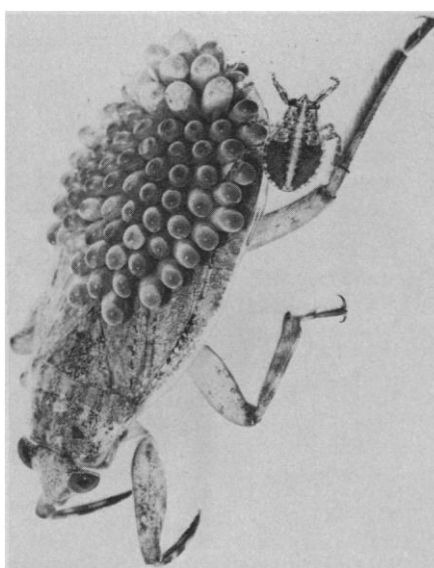

Fig. 1. Wild type (++) vasectomized male with eggs. Eleven swollen eggs on posterior fertilized by a homozygous striped male; not the brooder. Striped nymph recently hatched from one egg.

degree of paternity assurance would seem requisite to the evolution and elaboration of male brooding patterns. Given sperm storage by females, paternity assurance mechanisms in this case would necessarily involve competition between or among ejaculates from two or more males.

Such mechanisms seem to occur in the mating behavior of *A. herberti* (*5, 6*). Multiple bouts of copulation always preceded oviposition at male insistence, and copulation and oviposition were cyclical and male-controlled events. Males allowed remarkably consistent time periods for oviposition which were abruptly terminated by repeated coupling. Three eggs were the maximum number laid between bouts of copulation. In the extreme case, a pair coupled over 100 times in 36 hours during transfer of 144 eggs (*6*).

Sperm competition studies have been conducted on a variety of insect species representing several orders (*7, 8*). Of those studied, more than 75 percent exhibited sperm displacement; that is, sperm from the last male to mate predominated in fertilization of subsequently laid eggs. Although no sperm competition studies have been conducted on any Heteroptera, I predicted that sperm displacement or precedence should occur in *A. herberti* because it would provide a male with at least some degree of paternity assurance.

Several lines of evidence suggest that female *A. herberti* retain viable sperm from previous matings. Six of ten gravid females from a natural population near Phoenix, Arizona, had motile sperm in their spermathecae (*6*). Fifteen virgin *A. herberti* females were mated and allowed to deposit their entire complement of eggs on the backs of their respective mates. These females were killed and dissected in randomly selected groups of three at 30, 60, 90, and 120 days after mating. Two died between 120 and 150 days, but the remaining individual was killed and examined 150 days after mating. All 13 spermathecae contained motile sperm. Therefore, a male that receives eggs from a female previously mated up to 5 months before might risk having eggs fertilized by the female's previous mate or mates.

One can only infer risk from the presence of motile sperm in the spermathecae of nonvirgin females. Indisputable evidence would exist if eggs hatched from the backs of sterilized males paired with nonvirgin females. I vasectomized (*9*) a wild-type (++) male and paired him with a ++ female previously mated (30