Genomic Imprinting

Some Interesting Implications for the Evolution of Social Behaviour

Raghavendra Gadagkar

Raghavendra Gadagkar, who studies and teaches animal behaviour at the Centre for Ecological Sciences, Indian Institute of Science, and Jawaharlal Nehru Centre for Advanced Scientific Research, has a particular fascination for social insects such as ants, bees and wasps. He hopes to provide a Darwinian explanation for the altruism, courage, industry, sacrifice and such other seemingly human attributes of these insects.

In all diploid organisms such as ourselves, each individual inherits one set of chromosomes from the mother and another set from the father. It is generally assumed that once these chromosomes reach our bodies, they lose any 'memory' of where they came from. However there is evidence that chromosomes (and the genes they contain) sometimes get differentially imprinted as they pass through a male or female body and this imprint may be retained when the chromosomes are passed on to the next generation (see accompanying article by Ranganath and Tanuja). There is also evidence that DNA methylation is a mechanism by which chromosomes may acquire such male-specific or female-specific imprints. Differential patterns of DNA methylation are known to lead to different levels of gene expression. What all this means then is that our paternally derived genes and maternally derived genes may behave differently in our bodies even though they may be otherwise identical. To the extent that genes influence our behaviour it may well be that our father's genes and mother's genes pull us in different directions.

Sociobiological Theory

Although genes influence many kinds of behaviour, their influence on social behaviour is most relevant in the present context. This branch of study, sometimes called sociobiology, makes a number of predictions about how social behaviours evolve through the action of natural selection, even though the behaviours may sometimes appear to reduce the classical Darwinian fitness of the actors. All predictions of sociobiological theory are however based on the assumption that paternal and maternal genes do not behave differently. As early as 1982,

David Haig, then at the University of Oxford, pointed out that, if paternal and maternal genes did behave differently, many of the predictions of sociobiology would have to be reexamined.

Let us consider two examples of what might be called standard sociobiological predictions. In insects that belong to the order Hymenoptera (ants, bees, wasps) females can lay both unfertilized, haploid eggs as well as fertilized, diploid eggs. The fertilized diploid eggs develop into diploid adult females whereas the unfertilized haploid eggs develop into haploid adult males. Since males are haploid, they produce sperm that are clones of each other. The females, being diploid, produce haploid eggs that receive a randomly chosen 50% of the maternal genome. In such haplodiploid insects, two sisters would be related to each other by a coefficient of genetic relatedness r of 0.75 but a female would be related to her offspring by the usual 0.5 (as in diploid species) (*Figure* 1). In 1964 W D Hamilton pointed out that such asymmetries in genetic relatedness should select for altruistic behaviour on the part of females to care for their sisters rather than to produce their own offspring. This is indeed what workers (who are females) in many social insect colonies do. In 1976, Trivers and Hare pointed out that although workers are more closely related to their sisters ($r = 0.75$) they are much less related to their brothers ($r = 0.25$), as compared to their offspring ($r = 0.5$). They predicted therefore that either workers should prefer their own sons over their brothers or, if they are forced to rear their sisters and brothers, they should prefer to invest in their sisters and brothers in the ratio 3:1 (0.75 : 0.25). A particularly fascinating aspect of this prediction is that the workers' preferred ratio of investment (3:1) is in conflict with the queen's preferred ratio of investment of 1:1 in her daughters and sons. Hamilton's prediction and the prediction of

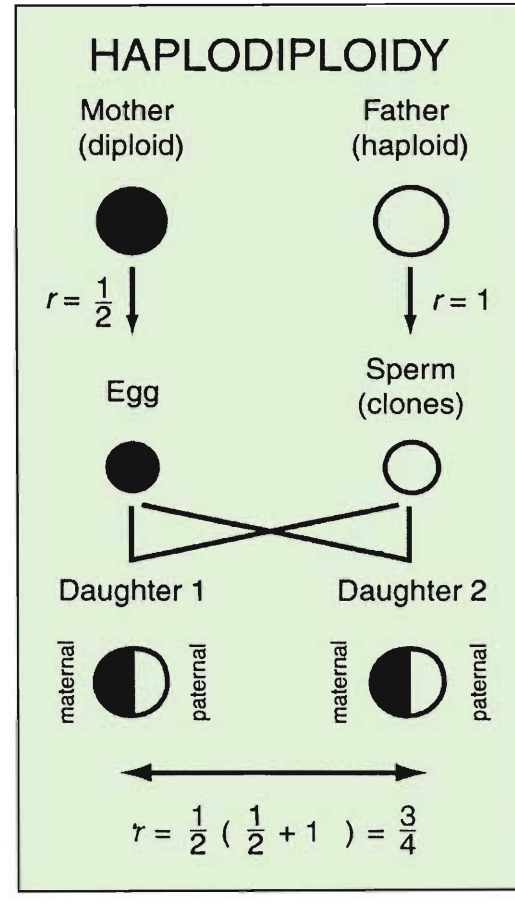


Figure 1. Genetic relatedness under haplodiploidy indicating how full sisters would be related by 0.75, rather than by the usual value of 0.5, seen in diploid species (see text for details).

Hamilton as well as Trivers and Hare computed relatedness values by taking the average values for maternal and paternal genes. But if the relatedness values are computed separately for the maternal and paternal genes, they turn out to be quite different.

Trivers and Hare have since become the cornerstones of sociobiology and both have engendered an enormous body of theoretical and empirical work.

Both these sets of predictions were however based on the assumption that maternal and paternal genes in the bodies of the worker behave identically. Thus Hamilton as well as Trivers and Hare computed relatedness values (0.75, 0.5 and 0.25, discussed above) by taking the average values for maternal and paternal genes. But if the relatedness values are computed separately for the maternal and paternal genes, they turn out to be quite different and so do the predictions. For example, from the point of view of the maternal genes in a workers' body, sisters are as valuable as daughters, so that altruistic rearing of sisters should be favoured no more than selfish rearing of daughters (in both cases, $r = 0.5$). From the point of view of the paternal genes on the other hand, sisters are twice as valuable as daughters so that altruistic rearing of sisters should be even more strongly favoured than selfish rearing of daughters (*Box* 1). A similar situation occurs with the predicted sex investment ratios. From the point of view of the maternal genes in a workers' body, sisters are as valuable as brothers so that a 1:1 sex investment ratio is favoured and thus there should be no conflict between queens and workers over sex investment. From the point of view of the workers' paternal genes however, all the paternal genes are expected to be found in sisters while none are expected to be found in her brothers. Hence paternal genes should favor all investment in sisters and none in brothers. Therefore queen-worker conflict should now be even more severe than what was predicted by a computation of average relatedness for maternal and paternal genes (*Box* 2).

Sex Determination in the Insect Order Hymenoptera

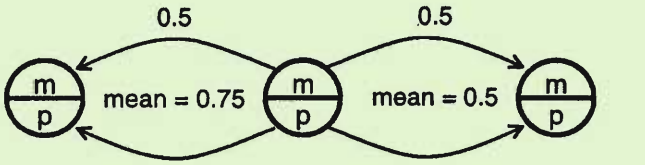
As David Haig, the man who originally raised the spectre of potentially overturning the predictions of sociobiological theory readily admits, whether a major reappraisal of sociobiological theory is required will depend on how common genomic im-

Box 1. Implications of Genomic Imprinting I (after Haig [6])

Intra-genomic conflict between maternal and paternal genes over selfishness versus altruism.

Hamilton's haplodiploidy hypothesis showing preference for sisters over daughters was based on average relatedness.

Hymenopteran colony with singly mated queen



Sister **1.0** **Worker** **0.5** **Daughter**

Average:	Sisters > Daughters (1.5 : 1)
Maternal genes:	Sisters = Daughters
Paternal genes:	Sisters > Daughters (2 : 1)

- Mild selection for rearing sisters instead of daughters (= social evolution)
- No social evolution if maternal genes are in control
- Rapid social evolution if paternal genes are in control

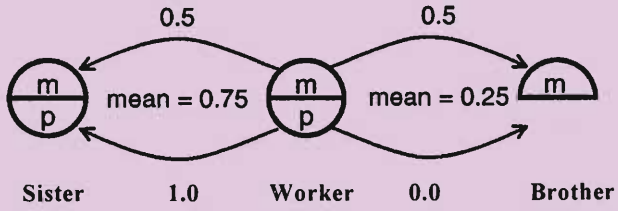
printing turns out to be in social insects. It is in this context that recent evidence for the role of genomic imprinting in sex determination in a parasitic wasp assumes significance. The wasp in question is *Nasonia vitripennis* and the study under consideration is by Dobson and Tanouye of the University of California at Berkeley. *Nasonia vitripennis* is a parasitoid wasp that is distributed throughout the world. Female wasps lay eggs in the pupae of flies that breed in carcasses and in bird nests. Like all hymenopterans, *N. vitripennis* is also haplodiploid and it is used as a favourite laboratory model system in a variety of genetic and evolutionary studies. As it often happens with laboratory model systems, many unusual mutants that cannot usually survive in nature turn up in the laboratory cultures. Many strains of *N. vitripennis* are now known that distort the sex ratio of their offspring – variously called son killers and daughter killers! A rather famous one is called PSR, for paternally transmitted sex

Box 2. Implications of Genomic Imprinting II (after Haig [6])

Intra-genomic conflict between maternal and paternal genes over sex investment ratio.

Trivers and Hare's prediction that workers prefer a 3:1 investment in their sisters and brothers was based on average relatedness.

Hymenopteran colony with singly mated queen



Average: Sister : Brother :: 3 : 1
Maternal genes: Sister : Brother :: 1 : 1
Paternal genes: Sister : Brother :: 1 : 0

- Some queen-worker conflict if mean relatedness matters (as queen prefers 1 : 1)
- No queen-worker conflict if maternal genes are in control
- Intense queen-worker conflict if paternal genes are in control

ratio factor. Unlike the wild type strains, eggs fertilized by PSR males also develop into haploid males but these males do inherit the PSR factor (*Figure* 2). It turns out that PSR is a small, aberrant, unpaired chromosome (such chromosomes are called *B* chromosomes) that enters the egg along with the paternal chromosomes. Having done so it brings about the heterochromatization and hence the loss of all paternal chromosomes. This leaves the zygote only with the maternal chromosomes and the PSR itself (*Figure* 3). Not surprisingly, such zygotes develop into haploid, PSR containing males. PSR has thus been dubbed the most selfish genetic element known. It uses the male to reach the zygote and having done so it destroys it's co-travellers (the paternal chromosomes) in order to ensure its future survival. PSR cannot survive unless it converts the diploid zygote that would normally develop into a female, into a male because PSR cannot transmit through a female body. PSR

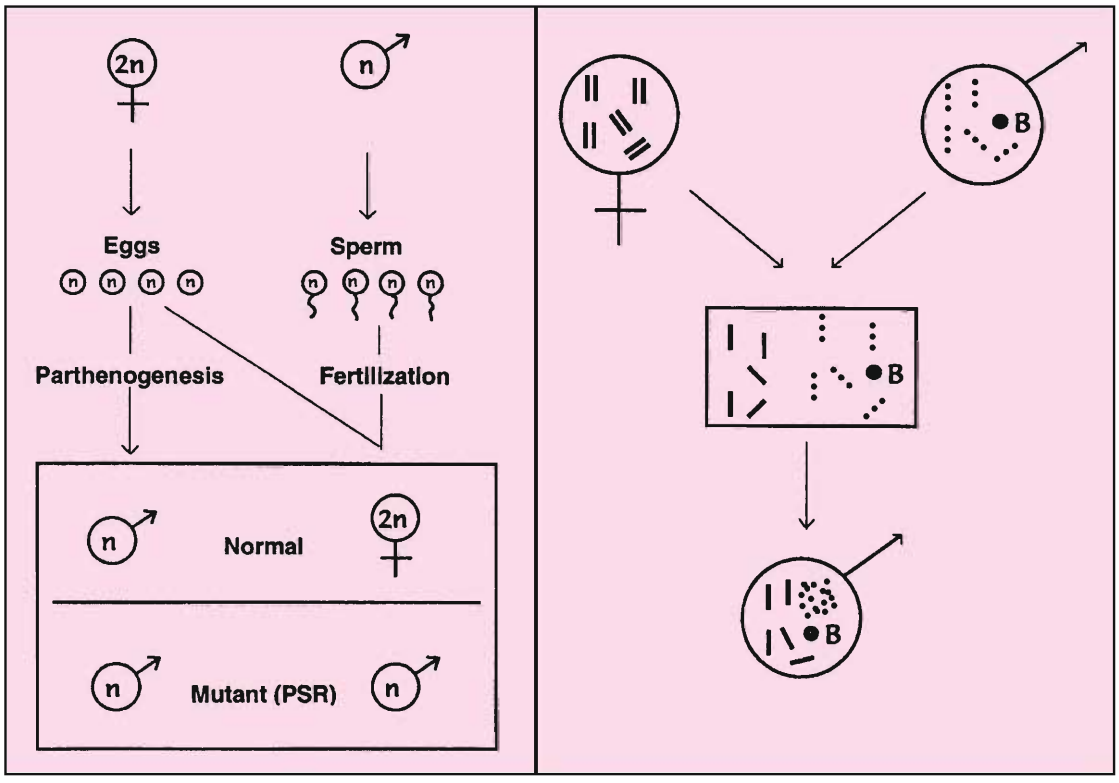


Figure 2 (left). Life cycle of normal and PSR strains of Nasonia vitripennis (after Werren and others [9]) (see text for details).

Figure 3 (right). The mechanism of action of PSR in converting fertilized eggs into males (after Nur and others [10]) (see text for details).

can only survive in a male body and since male hymenopterans normally have no sons it cannot survive unless it converts potential daughters into sons.

The aim of the Dobson and Tanouye study was to understand the mechanism of sex determination in the Hymenoptera. Even though we know that unfertilized eggs develop into males and fertilized eggs develop into females, the mechanism by which sex is determined is far from clear. The observation that unfertilized eggs develop into males and fertilized eggs develop into females is consistent with a variety of mechanisms – indeed there have been a variety of models proposed for sex determination in the Hymenoptera.

1. *Fertilization sex determination (FSD):* According to this model, the very act of fertilization causes the egg to develop into a female, quite independent of the paternal genes that fertilization may bring with it.

The observation that haploid eggs develop into males and diploid eggs develop into females is consistent with a variety of mechanisms of sex determination in the Hymenoptera.

2. ***Single locus complementary sex determination (SCSD):*** A single sex determining locus is postulated and individuals homozygous or hemizygous (as all haploid individuals are) are expected to develop into males while those heterozygous are expected to develop into females. Because the sex determining locus is believed to be highly polymorphic (having many alleles per locus), diploid homozygotes are expected to be rare and the usual way to get males is therefore by the development of unfertilized (hemizygous) eggs. As predicted by the model, diploid, homozygous males can be produced by inbreeding.

3. ***Multiple loci complementary sex determination (MCSD):*** Because the predictions of SCSD do not always fit the empirical data, multiple sex determination loci have been postulated for some species. The prediction is that individuals homozygous or hemizygous at all of these loci will develop into males while those heterozygous for any one of these loci will develop into females.

4. ***Genic balance sex determination (GBSD):*** According to this model, sex is determined by a balance between male determining genes (M) and female determining genes (F). Because M is postulated to be more powerful than F, haploid eggs with one set of M and F each develop into males (M > F). However fertilized eggs will have 2M and 2F. The M genes are not expected to be additive in their effects while the F genes are expected to be additive. Thus 2F > M > F, so that fertilized eggs develop into females.

5. ***Maternal effect sex determination (MESD):*** This model proposes that sex is determined by the ratio of nuclear and cytoplasmic factors. Haploid eggs, having one set of nuclear and cytoplasmic factors each, develop into males. Fertilized eggs, with one set of cytoplasmic factors and two sets of nuclear factors (one set received from the father), develop into females.

6. ***Genomic imprinting sex determination (GISD):*** This model proposes that genes in the mother are so imprinted that they can

only direct male development. However genes in the father are so imprinted that they can direct female development in spite of the presence of the maternal chromosomes. Only fertilized eggs contain genes with the paternal imprint and thus they develop into females.

New Evidence for Genomic Imprinting Sex Determination

It is fair to say that there is no satisfactory empirical support for any of these models. Genomic imprinting sex determination is the most recent proposal and the study of Dobson and Tanouye makes it possible to exclude all the previous five models and support only GIST, for *N. vitripennis*. Several other features of *N. vitripennis* permitted Dobson and Tanouye to design experiments that are not usually possible with other organisms. I have already described the PSR factor. By appropriate manipulations, one can also produce triploid females (with three sets of chromosomes) and diploid males. The triploid females produce haploid as well as diploid eggs and the diploid males produce diploid sperms! The basic experimental design of Dobson and Tanouye involved fertilizing haploid and diploid eggs with haploid and diploid sperm, with and without the PSR factor. With the help of three recessive eye colour markers they were able to assess whether the proportions of progeny of each sex and eye colour were as expected by different models for sex determination. Their most important result can be stated simply. When diploid eggs were fertilized by PSR containing sperm, the paternal chromosomes were lost as expected, leaving an embryo with two sets of maternal chromosomes and the PSR factor. All previous models of sex determination predict that these fertilized, diploid embryos should develop into females. They are fertilized (satisfying FSD), they are diploid and heterozygous (satisfying SCSD and MCSD) and diploid (satisfying GBSD and MESD). Only the genomic model predicts that these embryos should develop into males. And Dobson and Tanouye found that these embryos indeed developed into males (*Table* 1). As they admit, the possibility that PSR itself has male determining

It is fair to say that there is no satisfactory empirical support for any of the proposed models of sex determination in the Hymenoptera. The study by Dobson and Tanouye however makes it possible to exclude all previous models and support only genomic imprinting sex determination.

	Haploid eggs			Diploid eggs		
	Maternal chromosomes	Paternal Chromosomes	Sex	Maternal Chromosomes	Paternal Chromosomes	Sex
Unfertilized	One set	Nil	Male	Two sets	Nil	Male
Fertilized by wild type male	One set	One set	Female	Two sets	One set	Female
Fertilized by PSR male	One set	Only PSR factor	Male	Two sets	Only PSR factor	**Male**

Table 1. Sex determination in Nasonia vitripennis (after Haig [7], and Dobson and Tanouye [2].

genes cannot be completely ruled out. However previous deletion analysis (a technique by which various portions of the chromosome are deleted and then, by observing the resulting phenotype, one infers the function of the deleted portion) has failed to separate the ability of PSR to convert fertilized embryos into males and its property of eliminating paternal chromosomes. Thus it appears that elimination of paternal chromosomes is the mechanism by which PSR converts fertilized eggs into males. Although these eggs are fertilized and have a heterozygous, diploid chromosome composition, they only have maternally imprinted chromosomes. Lack of paternally imprinted chromosomes can thus be thought of as the reason why they do not develop into females.

Broader Implication outside the Hymenoptera

This evidence in favour of genomic imprinting comes as a reminder that a reappraisal of the sociobiological theory may be required sooner or later. The best evidence for the role of genomic imprinting followed by differential expression of maternal and paternal genes, comes from mammalian systems. And it is in mammalian systems that the role of genomic imprinting is also being vigorously investigated in another area of sociobiological theory namely, inter-sexual conflict. When

females mate with a different male each time they produce an offspring, male-female conflict can continue in the bodies of their offspring. While the mother would be selected (by natural selection), to distribute her resources nearly equally between her present and future offspring, the father would be selected to help the present offspring (which is his) to get as much of the maternal resources as possible, unmindful of the health of future offspring (who are not likely to be his). It has therefore been postulated that genes which may be involved in modulating the resource drawing abilities of offspring become differentially imprinted, to express the conflicting interests of the mother and the father. The most famous example is the case, or as Haig and Graham call it, "the strange case of the insulin-like growth factor II". Insulin-like growth factor II (IGF II) is a polypeptide that helps rapid embryonic growth in mice. As expected from the theory of genomic imprinting mentioned above, the paternal copy of IGF II is well transcribed while the maternal copy is almost silent. This is consistent with the idea that the father's genes are attempting to enhance the resource drawing ability of the offspring while the mother's genes are not particularly encouraging this. The 'strange' case concerns the type 2 receptor for IGF II. While the type 1 receptor appears to behave normally, the type 2 receptor is unusual. First it is transcribed mainly from the maternal genome and not from the paternal genome. Secondly the type 2 receptor has a very different function in other contexts: it is a cation-independent mannose-6-phosphate receptor which binds mannose-6-phosphate residues on lysosomal enzymes and transports them into lysosomes. Haig and Graham have theorized that the receptor which mediates the normal function of IGF II is the type 1 receptor and that the type 2 receptor has been hijacked by the mother to act as a sink for excess IGF II and thus limit embryonic growth. That the type 2 receptor gene is subject to imprinting of the opposite kind as compared to IGF II is consistent with this idea. Thus the father's genes appear to make plenty of growth factor and promote embryonic growth while the mother's genes find a way of eating up this growth factor and limit embryonic growth.

Conclusion

Admittedly, a great deal of all of this speculation remains to be tested, either by modelling or by experiments. But it is today's speculation that will guide tomorrow's research. If genomic imprinting turns out to be more common than is currently evident, then there is no escape from a major reexamination of many sociobiological predictions. And that would certainly cause a major turmoil or a great deal of excitement, depending on how you look at it!

Suggested Reading

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Address for Correspondence
Raghavendra Gadagkar
Centre for Ecological Sciences
Indian Institute of Science
Bangalore 560 012, India.
Email: ragh@ces.iisc.ernet.in
ULR: http://ces.iisc.ernet.in/hpg/ragh

"If one insists on looking for 'purpose in life', one might as well be satisfied with the realization that 'life' appears to anticipate a whole set of possible conditions which may or may not arise and that 'it' gets ready for them by making the necessary provisions by way of genetic 'freaks' which, if not much use at one time, may touch off the most useful genetic re-groupings at dramatic periods of large-scale environmental change. To put the matter paradoxically, it is only the freak who has a future and the blessings of the fully 'adjusted' organism cannot last longer than the context within which they are studied."

Max Hamburgh