Monozygotic twinning: An evolutionary hypothesis
(polyembryony/dizygotic/parent-offspring conflict/parental care/life histories)

SCOTT K. GLEESON†, ANNE BARRETT CLARK†, AND LEE ALAN DUGATKIN†

1School of Biological Sciences and Center for Ecology, Evolution and Behavior, University of Kentucky, Lexington, KY 40566; and 2Department of Biology, PO Box 6000, State University of New York, Binghamton, NY 13902

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ABSTRACT Monozygotic twinning is rare within populations yet taxonomically widespread. We explore the evolution of monozygotic twinning by modeling an allele in a newly formed offspring that causes it to undergo mitosis and separation to form one or more clones (twins), potentially in conflict with the parents’ interest. The success of this twinning allele in our haploid models depends on the balance of the benefit of increased frequency in the clutch and the cost of reduced survival resulting from limited parental resources. The trait reaches high frequency in a broad range of plausible conditions but also fails to spread or is kept at low frequency in others when the survival cost is high (e.g., in small clutch sizes). Interestingly, there are two reasonable conditions that predict high frequency of the trait but low visibility: random parental abortion and selection for low penetrance. Thus our models suggest reasons why monozygotic twinning might be rare, or alternatively, be common yet appear rare. In addition, we discuss the implications for sex-linked twinning, dizygotic twinning, and twinning by gametes.

Multiple births in humans has been the subject of myths in many cultures, as well as extensive scientific study (1-5). The rarity of such events, and the often negative effects on the well-being of the children or mother, contribute to general interest, while the unique genetic and social relationship between the siblings provides a compelling natural experiment for medical and psychological research. In evolutionary biology, on the other hand, the question of multiple births in any organism is a question of clutch size evolution, a central theme of life history theory. The theory of clutch size evolution has been cast primarily in terms of the trade-off between offspring number and offspring success (6, 7), as well as parental survival (8, 9). Clutch size is assumed to be under the control of parents, who are selected to adjust it to maximize their benefit. Evolutionary analyses of twinning patterns in humans have followed this framework: while increases in human clutch size might currently increase parental fitness (2), such increases in our recent evolutionary past would have reduced fitness because of reduced survival of the offspring (10). In keeping with this approach, it has been suggested that production of excess eggs, and the subsequent abortion of unwanted offspring, might be a form of bet hedging against fertilization failure (11); occasional errors in this mechanism may result in twinning as a non-adaptive accident (12). These hypotheses seem particularly appropriate to dizygotic twinning or more generally any increase in fertilizable ova released or zygotes successfully implanted during reproduction.

The expectation that existing clutch sizes usually reflect the optimal clutch size for the parent is complicated by the concept of parent–offspring conflict (13–17). Natural selection may often result in offspring trying to extract more investment from the parent than the parent is selected to provide. In this context, Williams (18) suggested that monozygotic twinning may be a trait expressed by the offspring in its own best interest, potentially in conflict with the interests of the parents. Twinning by offspring has the potential to alter the parents’ investment in a particular genotype and their final clutch size. In this paper, we present and discuss a simple model of the evolution of monozygotic twinning in a population of haploid organisms.

Twinning Models

The General Model. We assume that a gene for twinning evolves initially in a clutch determined by the parent. After fertilization, but before any significant parental investment (P1, defined as effort per offspring; ref. 19) has occurred, any members of the clutch that carry the twinning allele replicate asexually. This increases the frequency of the twinning genotype in the clutch and also increases the absolute number of offspring. If there are no opposing selective forces, the twinning trait will go rapidly to fixation. Furthermore, any tendency to produce triplets rather than twins will replace twinning, quadruplets will replace triplets, and so forth. However, some decrease in the fitness of each offspring with increasing clutch size is likely if we assume that parental effort (PE; ref. 20) is limited to some fixed total.

To model these ideas, we assume haploid sexual reproduction, clutches with a single pair of parents, random mating, and discrete generations. In the parental population, the frequency of the wild-type nontwining allele at time t is p and that of the twinning mutant allele (henceforth the tw allele) is q. Assuming no sampling error (see below), each parent of a pair contributes half of the genes to a clutch size of n haploid offspring. Each tw-carrying offspring in the clutch will replicate itself by some factor z, where z can be 2, 3, 4, etc. Thus “twinning” will be used broadly here to indicate any amount of self-replication by an offspring. In addition, fractional values of z can be interpreted as partial penetration (see below).

As clutches increase above n, there will be declines in offspring fitness, with fitness defined as the probability of survival to the age of average reproduction. We define s0 as the fitness of each offspring in pure wild-type clutches and normalize it to s0 = 1. The relative fitnesses of offspring from clutches where one parent carries tw or both parents carry tw are s1 and s2, respectively. The proportion of tw in the next generation will equal the twedtw offspring surviving from mixed broods and pure tw broods as a fraction of total offspring produced and surviving from all broods, i.e.,

\[ q_{t+1} = \frac{nz(pqs_1 + q^2s_2)}{np^2 + npqs_1 + npqs_2 + nq^2sz} \tag{1a} \]

which reduces to

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For a rare tw allele to increase in the second generation, it must have a positive rate of increase ($\Delta q = q_{t+1} - q_t > 0$) when $q$ is near zero. This condition is satisfied when the fitness ($w = s_1z$) in mixed-parnterage clutches exceeds the fitness of offspring in pure wild-type parent clutches ($w = 1$)—i.e., $s_1z > 1$, or $s_1 > 1/z$. For example, if twinning is taken as $z = 2$, then the reduction in fitness for tw offspring in the mixed broods must be $<50\%$. In this model, $n$ cancels out of the equations, and $tw$ evolves independent of starting clutch size and the absolute increase in clutch size. This is because the allele frequency is sensitive only to proportional changes within clutches, which are predictable on the basis of parental genotypes. Clutch size will, however, affect twinning evolution by causing sampling error in small clutches (see below).

How much tw will spread—i.e., its equilibrium frequency ($\Delta q = 0$)—will depend not only on $s_1$ and $z$ but also on $s_2$, the fitness in pure tw clutches. The equilibrium level attained by a rare twinning allele will vary with the values of $s_1$ and $s_2$ as shown in Fig. 1 for $z = 2$. Positive values occur when $s_1 > 1/z$ and equilibrium $q$ increases with increasing values of $s_2$.

There are two obvious ways in which parents might reduce individual offspring survival in twinning clutches: random embryo abortion immediately after twinning to reestablish the parental optimum clutch size or a reduction in investment per offspring in enlarged clutches. While not mutually exclusive, these two parental responses have rather different implications for the parents' fitness as well as for the fitness of the twinning offspring.

**Parental Control over Clutch Size (Model 1).** After twinning and the concomitant increase in clutch size, but before any significant investment, parents decrease the clutch size to their original optimum, $n^*$, by abortion, limitation of implantation sites or nest space, or preemptive egg reduction. If the culling is random with respect to offspring genotype, then the per-twinning chance of survival is proportional to the amount by which the clutch is increased, i.e., $s = (\text{optimal clutch size} n^*)/(\text{enlarged clutch size})$. Clutches with wild-type parents don't change and produce $n$ offspring ($s_0 = 1$). Clutches with two tw parents increase and are reduced back to $n$, still with 100% frequency of tw ($s_2 = 1/z$). In the wild-type $\times tw$ clutches, per-twinning survival goes down as the magnitude of twinning (value of $z$) increases [$s_1 = 2/(1 + z)$], but the fraction of survivors that are tw goes up $[z/(1 + z)]$. As a result of this advantage in mixed broods, tw will spread whenever it arises. When these values are substituted into the basic model (Eq. 1b), it reduces to $q_{t+1} = pq(2z/(1 + z)) + q^2$ and $\Delta q = pq(z - 1)/(z + 1)$, so the twinning gene will increase ($\Delta q > 0$) when $z > 1$ and will go to fixation ($\Delta q = 0$ as $q \rightarrow 1$). As noted, there is no frequency gain due to twinning in pure twinning clutches. The process is driven entirely by the frequency advantage of twinning in mixed clutches, an advantage that grows larger as $z$ increases. Selection thus maximizes $z$. While twinning is strongly selected for in this model, it has no effect on final clutch size and will be inconspicuous.

**Reduction in Parental Investment (Model 2).** Parents that cannot reduce the number of young in a clutch but still have an upper limit on the parental effort for a clutch (PE) might reduce the investment per offspring (PI). Assuming that the parent allocates resources unselectively (i.e., $PI = PE/n$), there will be some functional relationship between offspring survival, parental investment, and clutch size. One simple relationship would be a monotonic decline in per-twinning survival ($s$) with increasing clutch size ($n$); for example,

$$s = \frac{x + 1}{n_{\text{max}}} - \frac{n^2}{n_{\text{max}}^2},$$

where $n_{\text{max}}$ is the clutch size at which offspring survival declines to zero, and $x$ is an exponent that controls the curvature of the function, with $x = 1$ a straight line. The function is concave upward when $x < 1$ and concave downward when $x > 1$.

From the parents' point of view, reducing per-twinning investment differs substantially from controlling clutch size. Without the option to abort, the parent is forced to deviate from its optimum whenever it has tw-carrying offspring in its brood. The parental optimum is important in that it sets the initial brood size and thus it controls the fitness consequences of twinning (16). With discrete generations, the parental optimum $n^*$ is reached when $dn)/dn = 0 (6)$ and is $n_{\text{max}}/(1 + x)^{1/2}$. In the linear case ($x = 1$), this reduces to $n_{\text{max}}/2$. Eq. 2 was constructed to normalize offspring survival under the parental optimum to $s_0 = 1$ so that it may be incorporated directly into the genetic model.

Initial increase of the twinning allele depends on its success in mixed clutches ($w = s_1z$). This can be solved in the linear case ($x = 1$). If $n^*$ is the optimal parental clutch, then $n_{\text{max}} = 2n^*$. In a mixed clutch, since half the clutch will be twiners, the new clutch size will be $n = n^*(z + 1)/2$. From Eq. 2, it follows that $s_1 = (3 - z)/2$. For $z = 2$, $s_1 = 0.5$ and $w = s_1z = 1$. Thus, for a linear function and $z = 2$, tw is neutral when rare because the twinner in a mixed clutch has the same fitness as an offspring in a nonwinning clutch. Furthermore, it will be selected against if it increases in frequency, because its survival in the resulting pure tw clutches is zero ($s_2 = 2 - z = 0$). For twinning of $z = 2$ to evolve, there must be some upward concavity of the fitness function (i.e., $x < 1$). If Fig. 2 illustrates the effects of the shape of the fitness function ($x = 0.1, 1, 10$) and different magnitudes of twinning ($z$) on the expected frequency of tw at equilibrium. The lower the increase in survival cost with increases in clutch size (lower values of $x$), the more successful are the twinning alleles.

**Penetrance.** Williams (18) speculated that twinning would be less costly to the parent in larger litters because the effect of one twin would decrease as clutch size increased. Clutch size had no effect on the likelihood of twinning in our model, however, because when twinning is inherited from the parents the proportion of twins is determined rather than an
In the clutch size control case (model 1), there is no longer a single $s_1$ for wild-type and $tw$ alleles in mixed clutches. Culling produces a survival rate for the wild-type of $s_{1w} = n/n_r$ and a survival rate for the twinner of $s_{1z} = n/n_r$. As before, $s_2 = 1/z$. The frequency of the $tw$ allele is then

$$q_{t+1} = \frac{pq s_1 z + q^2}{p^2 + pq(s_{1w} + s_{1z})z + q^2}.$$  \[3\]

As shown in Fig. 3A, $q$ increases and $tw$ goes to fixation (when $n > 1$), but it does so more slowly with smaller initial clutch sizes.

In the case of parental investment reduction (model 2), the subjective clutch sizes can be substituted into the fitness function (Eq. 2) to determine the fitness of each genotype in mixed clutches (Eq. 1). The subjective fitness approach assumes a linear fitness function so the results are only approximate for nonlinear functions. Fig. 3B shows the effects of varying initial clutch size in the linear case ($x = 1$) with $z = 1.5$. As clutch size gets smaller, the equilibrium frequency of the trait declines.

Clutch size has these suppressive effects because of the increased variance with smaller clutch sizes. The larger the variance, the stronger the self-suppression of the twinning alleles and the stronger the effects of between-clutch selection against the twinning phenotypes.

**DISCUSSION**

These models show that an allele promoting the twinning of offspring prior to parental investment could indeed spread. Yet data from humans indicate that monozygotic twinning is rare and not inheritable (2, 5). We suggest that the real interest of this model lies in its predictions about where twinning would or would not be found and how easily twinning alleles could be detected if they exist.

**Limits on the Spread of Twinning.** Twinning is often favored in the model because any reduction in fitness is applied evenly across all offspring, and in mixed broods the increase in the frequency of twinning alleles through replication is often sufficient to offset the reduced survival per offspring. The result is a reallocation of parental effort from the nontwinners to the twins.

We modeled two potential causes of survival reduction. With random abortion of offspring (model 1), not only is twinning strongly favored, but alleles that produce triplets, quadruplets, etc., are favored over those producing fewer duplicate young. However, such events might be hard to detect because they are expected to occur soon after fertilization, before implantation, and the final clutch size and sex ratio would go unaltered unless twinning were sex-biased (see below). The results of this mechanism would be similar to the

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**Fig. 3.** Inhibition of the twinning allele in small clutches including sampling error in model 1 (A) and model 2 (B), where $n$ refers to the optimal parental clutch ($n = 5, 20$, and 1000). (A) In model 1 (random abortion, with $z = 2$), the allele still goes to fixation, but at a slower rate. (B) In model 2 (reduced parental investment, $z = 3$, $z = 1.5$), the allele is slowed and the equilibrium frequency is reduced.
“vanishing twin” phenomenon in humans (22) in which the prenatal twinning rate appears to be considerably higher than the rate observed at birth. When parents reduced investment without clutch reduction (model 2), twinning alleles did not always go to fixation or even succeed in the model, especially in large clutches. Equilibrium levels balance the increased frequency of twinning alleles in the brood and reduced survival with increasing clutch size. Levels of twinning between 1 and 2 were often successful and can be interpreted as alleles with low penetrance. Thus, the model suggests that positive selection for low rates of expression could result in high frequencies of the twinning alleles. With low penetrance and small clutches, twinning would be rare but very noticeable when it happened. Low penetrance in large clutches would make the trait practically unnoticeable, even when the twinning allele was more common.

In summary, there are clearly parameter values for the general model that directly prevent the evolution of twinning. However, the results also suggest several reasons why a common monozygotic twinning gene might appear both rare and nonheritable. First, nonsyntrophic parentally controlled mutation of “extra” offspring may mask any effects on clutch size variation per se. Second, if low penetrance is favored, the allele will be common but seldom expressed. Finally, because selection between clutches may inhibit the spread of the allele when clutch size is small, heritable twinning may be found mainly in large clutches where it is hardest to detect.

Alternate Assumptions. A number of additional or alternative assumptions could be incorporated. We have modeled the diploid case including multiple paternity of broods and evolutionarily stable conditions and found no qualitative effects on the results (the dominant diploid model has the same behavior as the haploid model described here). Incorporation of immediate survival costs to the twinning offspring (e.g., reduced size, malformation, conjoinment) has the predictable effect of inhibiting the trait. Further modifications of interest would include the effects of age structure, other forms of parental response, environmental variation, deleterious effects of reduced genetic variation (18), and the consequences for interactions between twins (23).

Other Applications. In this section, we consider implications of the hypothesis for sex ratio, dizygotic twinning, and gamete twinning. Sex ratio of the brood will be biased if the twinning allele is restricted to a single offspring in a clutch or if the twinning allele is sex linked or sex limited. If this results in higher intrabrood competition relative to a more even sex ratio, it should inhibit the trait, especially in small clutches. Alternatively, monozygotic twins may be more advantageous for one sex than the other. The sex with higher resource needs may find twinning more disadvantageous because it pushes the clutch closer to the maximum clutch size. The less competitive sex might benefit from twinning, especially if parents respond by culling the brood to their optimum size, leaving this sex with a less competitive environment. Sex-biased twinning as a parental mechanism for creating male-biased litters has been suggested for lions where male offspring benefit reproductive from having a number of male siblings (24). In this case, parent and offspring may not be in conflict, but sex-linked genes in the offspring could potentially evolve even if detrimental to the parent with these within-clutch selection pressures.

Dizygotic twinning might also be interpreted in terms of parent-offspring conflict. Sex-biased twinning with divergent self-interests are created in meiosis, so that all incipient eggs are in potential conflict with each other and the parent. Any egg that is produced but not released has higher potential fitness if it can manage to be released, even into a suboptimally large clutch. In an iteroparous species, an allele for early release might be favored if there is any likelihood of parent mortality. Thus normal dizygotic twinning, polyovulation, superfetation (release of eggs during gestation), and polar body twins (25–27) could all be expressions of gameteparent conflict.

Twinning of gametes prior to fertilization could result in a third type of twinning, “monozygotic twinning,” which would superficially appear to be dizygotic twinning. If an egg cell replicated and each mitotic product were then fertilized by different sperm, the result would be twins that superficially resembled dizygotic twins in physical appearance (including sex ratio but not spread by 1/4 rather than 1/2). The selective mechanism is identical to monozygotic twinning except that it is expressed only in maternal lines. Competition between sperm of the same individual for fertilization of the same egg might favor sperm twinning. Parental interest in maximizing sperm number may have already reduced sperm to some minimal viable size, which would inhibit such a trait, but this remains an open question (28, 29). If competition is for more than one egg, monoametic twins could be produced by twin sperm fertilizing eggs in the same litter.

Twinning Patterns. There is very little data on monozygotic twinning in animals other than humans and a few other groups with small litter sizes. In fact, since the primary method for detecting monozygotic twins has been to compare the sex ratios of twin births (30), only species that normally produce singletons have been tested.

Monozygotic twins are related as full sibs (mean coefficient of relatedness, r = 0.5) and are equally likely to be the same or different sexed. Dizygotic twinning shows some evidence of heritability, is both relatively high and variable in frequency within populations (0.2–2%), and increases with maternal condition and age (with a peak at age 3). In comparison, monozygotic twins are genetically identical (r = 1.0) and thus always the same sex. Such twinning shows less evidence of heritability or maternal effects and is relatively low in incidence and stable across populations (0.35%) (2, 5). However, there are some reports of unexpectedly high frequencies of monozygotic twins in certain family lineages (30–32). This led Harvey et al. (31) to propose “the possibility of a single gene dominant mode of transmission with variable penetrance as one mode of determination for monozygotic twinning.”

In nonhuman primates that normally bear singletons, twins have been observed to occur at a rate comparable to humans (33, 34). Those that regularly have two young (e.g., ring-tailed lemurs, callitrichids) apparently produce fraternal twins, based on analyses of the frequency of same-sex pairs (33, 34). Twinning in some domestic mammals with small litters has also been studied. In horses, twins are quite common (18%) and thought to be heritable but considered undesirable because the offspring suffer high mortality or other problems (37, 38). In other animals, especially cattle and sheep, twinning is seen as a way of improving production, and research efforts focus on increasing the trait by selection or artificial manipulation (39, 40). In cattle, twinning is known in animals that confine their offspring to eggs such as lizards and birds (43–46) and even worms (47). As Williams (18) noted, egg-laying poses a distinct problem for the evolution of twinning by offspring—if parental investment is restricted to the egg contents, it is effectively a clutch of n = 1 and monozygotic twinning within an egg would be disadvantageous to the tanner as well as the parent. Selection for
Evolution: Gleeson et al.