Do Norway rats (*Rattus norvegicus*) synchronize their estrous cycles?

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Abstract

Estrous synchrony was tested using 10 pairs of sibling female rats (*Rattus norvegicus*). A Monte Carlo bootstrap simulation was used to construct random control groups to avoid previous statistical errors and to test for significance when there are irregular cycles. The 10 pairs of females did not exhibit estrous synchrony. The effect of cycle irregularity on the limits of synchrony was analyzed using an equation that related the degree of cycle regularity to the degree of synchrony. This equation significantly predicted the limits of synchrony: cycle irregularity limits both the maximum and minimum degree of synchrony that can occur between two females. Finally, simulations of the expected levels of synchrony in groups of five rats were compared to the original study on estrous synchrony. The simulations indicated that the results of the original study were consistent with chance levels of synchrony. It is concluded that there is no evidence that Norway rats synchronize their estrous cycles. Evolutionary implications are discussed.

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The report [18] of estrous synchrony among Norway rats (*Rattus norvegicus*) has become highly influential in menstrual synchrony and human pheromone research [21,22,40]. This finding motivated the development of a coupled-oscillator model [34] for the hypothesis that rats and other female mammals synchronize their ovarian cycles by releasing pheromones: one that reduces cycles (released during the follicular phase of the cycle) and the other that lengthens them (released during the ovulatory phase [20–22,40]). The coupled-oscillator model and hypothesis also provided the theoretical basis for a study [40] reporting the discovery of two pheromones that regulate the menstrual cycles of women in the same way as they regulate the estrous cycles of rats [22,40].

Methodological issues concerning the evidence for ovarian-cycle synchrony in other mammals, especially humans, have become increasingly salient. For women, while there are a number of studies that have reported menstrual synchrony [9–11,14,16,17,26,37,44–46,48,52,55,56], nearly one-third have failed to find synchrony [3,13,38,39,58,59,60]. The first alternative is virtually untestable, and although it may seem unusual that the existence of such a widely studied phenomenon is still under dispute, this does happen and for interesting methodological reasons — such as methodological problems with the evolution of “industrial melanism” in peppered moths [57].

Ovarian synchrony has also been reported in several other mammals: chimpanzees [43], golden lion tamarins [6], golden hamsters [12] (but not among Djungarian hamsters [4]). However, the synchrony reported among golden hamsters was likely due to pseudoentrainment (i.e., the likely chance coincidence of cycles given a criterion for detecting synchrony of four consecutive days of similar vaginal smears [30]). The statistics and methods used by Wallis [43] were flawed and biased [38], and French and Stirbley [6] used the same methods as Wallis with similarly flawed results that were not replicated in a subsequent study [24]. Specifically, Wallis [43] and French and Stirbley [6] used parametric statistics to analyze all possible pairings of individuals to assess cycle onset differences among individuals in a group. This inflated the degrees of freedom...
factorially as a function of group size, greatly increasing the likelihood that chance effects would be amplified to statistical significance (a Type I error). In addition, it has long been tacitly assumed that the expected mean onset difference between ovarian cycles of two females is one-fourth their mean cycle length. This assumption is in error whenever cycles are an odd number of days in length or there is cycle-length variability [32]. In either case, the expected mean cycle onset difference is less than one-fourth the mean cycle length, creating a bias towards detecting synchrony when it does not exist [32]. Thus, given the recent controversy concerning the very existence of ovarian synchrony in mammals and claims about its potential implications for human physiology, psychological states, social behavior, and fertility [22], this paper reexamines the phenomenon of estrous synchrony among Norway rats.

Returning to the coupled-oscillator model [34], it was and still is the only formal model ever developed to explain ovarian synchrony in mammals. According to this model, synchrony can occur in groups of two or more females [34]. Studies of menstrual synchrony have mainly focused on pairs of women (e.g., Refs. [17,48]). Thus, if a coupled-oscillator mechanism applies to both female rats and women [21,22,34,40], we should expect to find synchrony in pairs of female rats. Moreover, if synchrony between female rats occurs under conditions similar to that of women (i.e., among close friends or sisters who live together, e.g., Refs. [17,48]), then we should find synchrony among pairs of female rats who are sisters and are housed together.

This hypothesis was tested using 10 pairs of sibling female rats. Statistical analysis was performed by using a bootstrap Monte Carlo algorithm. This approach allowed the testing of this hypothesis even when some females have irregular cycles and to assess the degree of synchrony that is achieved or maintained over a period of time. The limits of synchrony were then analyzed as a function of the degree of cycle regularity. Finally, the original study [18] was reassessed with the help of computer simulation.

1. Methods

1.1. Subjects

Twenty non-mated Sprague–Dawley female rats (five groups of four sisters) were used; born and bred at Indiana University from stock originally obtained from Taconic (Germantown, NY). Animals were housed in standard polypropylene maternity cages (48 × 20 × 26 cm) and provided with food and water ad libitum. Colony rooms were maintained at 24°C ± 2°C and illuminated from 08:00 to 20:00 hours.

The females came from litters that were culled to four females and four males at 3 days of age (day of birth is day 0). All litters were weaned (as part of the standard laboratory protocol) at 30 days of age, the four sibling females from each litter were then housed together in standard maternity cages until 70 days of age. At 70 days of age, five groups of four females were moved to the experimental room. At that time, each group of four sisters was randomly separated into two groups of two sisters and housed in maternity cages. The experimental room was maintained at 24°C ± 2°C with relative humidity between 30% and 60%, illuminated from 08:00 to 20:00 hours, and differed from the main colony rooms in containing no other animals except the 10 experimental pairs. The procedures used were non-invasive and met the guidelines for animal care and use at Indiana University.

1.2. Vaginal smears and estrous cycles

Vaginal smears were collected between noon and 16:00 hours by daily saline vaginal lavage and classified immediately thereafter by estimating the relative proportion of three types of cells: cornified (C), nucleated (N), and leukocytic (L [36]). A smear class is the relative proportion of each cell type present in a smear with a threshold of 10% as the criterion for including a cell type [36]. Thus, there are 15 possible smear classes (i.e., C, CN, CNL, . . . , L, LN, LNC).

It is conventional to distinguish four states of the estrous cycle: estrous (E), metestrus (M), diestrus (D), and proestrus (P), each of which can be viewed as comprising 1 day of a 4-day estrous cycle (e.g., . . . , E, M, D, P, E, . . . ). Smear classes are associated with these four states of the rat estrous cycle. C and N smears (e.g., C, N, CNL) are associated with estrous and proestrus, and L-dominated smears (e.g., LN, LNC, LCN) are associated with metestrus and diestrus.

Knowledge of the smear class itself does not uniquely indicate the state of an estrous cycle [5]. The sequence of vaginal smear classes does provide this information and can be used to produce a better classification of estrous-cycle states. A smear is C dominated if C cells are the predominant cell type; N dominated if N cells are the predominant cell type, and there are less than 10% L cells; otherwise, it is an L smear. For example, NC would be N dominant, but NCL would not. The most reliable indicators of state change are the transitions from C- and N-dominated smears to L smears (L’s in the first or second position) and from L smears to C- and N-dominated smears [5].

The rules used to classify the states of the estrous cycle from vaginal smears were based on Everett [5] and listed in order of application (see Table 1): (i) An estrous cycle is a minimum of 3 days in length with estrous, metestrus, and proestrus. (ii) A transition from a C- or N-dominated smear (e.g., C, CN, NC, CNL) to an L smear (e.g., L, LN, LNC, NLC) indicates a transition from estrous to metestrus. (iii) A transition from an L smear to a C- or N-dominated smear indicates a transition from diestrus to proestrus. There are two exceptions: (a) if there is only one L smear since the last estrous, then the transition is from metestrus to proestrus and (b) if the smear following the C- or N-dominated smear is an L smear, then the transition is from proestrus to
estrous. (iv) A persistent estrous is more than one C- or N-dominated smear between proestrus and metestrus. (v) A persistent or prolonged diestrous cycle is more than three L smears between an estrous and proestrus.

1.3. Procedure

After females were moved to the experimental room, daily smearing commenced for 48 days and their vaginal smear classes were recorded. This is similar to the typical design of menstrual synchrony studies in which menstrual cycles are recorded over several months or cycles (e.g., Refs. [17,48]). At the end of 48 days, the vaginal smear classes were transformed into sequences of estrous-cycle states using the above rules. Not every smear class in the 48-day sequence could be classified using these rules because daily smears might start with a sequence of smears, which are indeterminate in their classification. For example, a sequence of . . . , LN, LNC, . . . is indeterminate because we do not know whether the LN was proceeded by a C- or N-dominated smear or another L smear (similar problems occur in the menstrual synchrony literature [60]). By applying rule (ii), however, we can classify the first transition from a C- or N-dominated smear to an L smear as E to M. Similarly, we can classify the last cycle transition. For each female, the first E to M transition marked the beginning of the first cycle and the first cycle begins with M. The last cycle was also marked by an E to

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**Table 1**

Examples of rules for classifying vaginal smears as estrous-cycle states

<table>
<thead>
<tr>
<th>Rule no.</th>
<th>Vaginal smear sequence</th>
<th>Estrous-cycle state sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>CN, LN, N, CN, LNC</td>
<td>E, M, P, E, M</td>
</tr>
<tr>
<td>ii</td>
<td>NC, LNC</td>
<td>E, M</td>
</tr>
<tr>
<td>iiia</td>
<td>CN, LNC, NC, C</td>
<td>E, M, P, E</td>
</tr>
<tr>
<td>iiib</td>
<td>LCN, C, LNC</td>
<td>P, E, M</td>
</tr>
<tr>
<td>iv</td>
<td>LNC, N, C, CN, LN</td>
<td>D, P, E, E, M</td>
</tr>
<tr>
<td>v</td>
<td>C, LN, LNC, LNC, LCN, NC, C, LNC</td>
<td>E, M, D, D, P, E, M</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Cycle sequences for the 10 pairs of rats. The estrous-cycle states are estrous (E), metestrus (M), diestrus (D), and proestrus (P). The arrows indicate closest estrous states (E) identifiable at the beginning and end of the 48-day observation period.
1.4. Statistical methods

When analyzing synchrony among individuals, it is important to distinguish a process of becoming synchronized from the level of synchrony achieved and maintained in a group [34]. During a process of synchronization, a group of individuals changes from its current level of synchrony to a higher but not necessarily maximum level of synchrony (i.e., maximum synchrony occurs when the states or phases of all individuals are in exact lock-step synchrony [62]). It is assumed here, as in the ovarian synchrony literature (e.g., Refs. [17,54]), that once a process of synchronization has occurred, a group of females should achieve a stable level of synchrony. Norway rats should achieve a detectable and stable level of synchrony in about 30 days [18].

First estrous occurs at about 36 days in these females [33], thus, by 70 days, most females had been cycling for at least 30 days and should have achieved a statistically detectable level of synchrony. When groups of females were moved to the experimental room, their grouping condition was changed from four to two in a cage, which may have changed the level of synchrony between pairs. If this produced an initial decrease in the level of synchrony, then we should have observed a process of synchronization over the following 48-day interval (as the pairs resynchronized their cycles). If no change in synchrony level occurred, then the degree of synchrony early in the observation period should have been the same as that towards the end.

To detect a change in the level of synchrony, a paired t test was performed by comparing the first and closest estrous-day differences with the last and closest estrous-day differences for each pair (see Fig. 1). If the level of synchrony did not change over the observation period, we still do not know whether the pairs of rats were initially at a level of synchrony greater than what would be expected by chance. To assess the level of synchrony, we must compare the observed level of synchrony with that expected by chance.

For regular cycling rats, the expected probability of estrous-cycle state matches over time is exactly determined by a multinomial equation [29]:

\[
P(n_1, n_2, \ldots, n_k) = \frac{k!}{\xi(s_1)! \cdots \xi(s_r)!} \times \left( \frac{N!}{n_1! \cdots n_k!} \right) \left( \frac{1}{k} \right)^N
\]

where \((n_1, n_2, \ldots, n_k)\) is a sequence representing the number of individuals in each of \(k\) states, where \(n_1 + n_2 + \ldots + n_k = N\). The term \(\xi(s_i)\) is the number of subsequences of states with exactly the same number of individuals in them. This type of synchrony has elsewhere been called \(Nk\) synchrony [29]. It assumes that all individuals cycle through exactly the same number of non-overlapping states, each state having the same duration in time.

Eq. (1) specifies probability distributions for the various combinations of \(N\) individuals distributed over \(k\) states at a given time. For a pair of individuals, either all states match or they do not, and so the match between two females with exactly the same cycle length is either 1 or 0. For \(m\) pairs of rats, the probability of \(r\) pairs matching is binomial and the probability, \(P\), of a match can be calculated using Eq. (1) with \(N=2\) and \(k=4\), yielding \(P=.25\). For \(m=10\), the binomial distribution for pairs of 4-day cycling rats is illustrated in Fig. 2.

If there is any cycle irregularity at all, Eq. (1) does not apply. Sprague-Dawley rats most frequently exhibit 4-day cycles followed in frequency by 5-day cycles, and cycles of longer length [19]. Longer cycles are up to 2 weeks in length [5], and may be more typical of wild rats [2,15,19]. Thus, information about long cycles should not be thrown out because we lack analytical methods for generating probability distributions for irregularly cycling animals. Instead, we need a general method for determining whether pairs of females exhibit a closer than random match between their estrous-cycle states over time.

A more general method is to use Monte Carlo bootstrap simulation (e.g., Refs. [28,42]). This is a generalization of the randomized control-group procedure used by McClintock [18]. By using a bootstrap approach, not only can we numerically estimate the special case of exact estrous-cycle state matching in 4-day cycling rats (see Fig. 2), but we can also calculate probability distributions for irregularly cycling rats.

A simple bootstrap algorithm for randomly pairing sequences of states is illustrated in Fig. 3. Imagine that the sequences of states a rat goes through during an estrous cycle are represented on a tape as in Fig 1. Two
tapes represent two sequences. We can calculate the frequency distribution of state matches for \( m \) pairs of rats by connecting each tape together (middle of Fig. 3) into a ring, randomly spinning each of them, and then cutting the tapes and laying them along side each other in the same relative positions as before. By repeating this over and over again, we get a frequency distribution of matches for the \( m \) pairs.

The physical metaphor depicted in Fig. 3 is easily programmed (e.g., by writing macros in Microsoft Excel). This bootstrap method makes no assumptions about the length of cycles or duration of states (in days), it simply repeatedly samples from the actual data. Thus, cycle irregularity does not block our ability to estimate the likelihood of the actual match observed. The statistic calculated is the mean frequency of match for the 10 pairs of estrous-cycle state sequences illustrated in Fig. 1. A match of 0 is minimum synchrony, while a match of 1 is maximum synchrony. From 10,000 simulated spinnings of the data (for all 10 pairs), a probability distribution was generated for estimating the likelihood of mean match observed. Significant matching of states must occur less than 5% of the time \((\alpha = .05)\).

2. Results

The level of synchrony from the first closest pair to the last closest pair remained the same (mean difference in days = 0.8, both at the beginning and end, \( t = 0, df = 9, P = 1 \), two-tailed; see Fig. 1 for the estrous state matches). This implies that moving the females to another room and separating them into pairs did not affect their level of synchrony over the 48-day observation period. The degree of match between pairs of females also did not differ from chance (mean match = 0.247, \( P > .62 \)). Fig. 4 illustrates the bootstrap-generated distribution of frequencies of matches. The actual degree of match is near the peak of the distribution as indicated by the arrow.

3. Discussion

No effect of synchrony was found, and the results were consistent with chance levels of synchrony. Failure to produce synchrony in a particular experiment does not disprove the phenomenon of synchrony. Indeed, one cannot rule out the possibility of a Type II error given the small expected effect size of synchrony previously reported [18,34]. However, assessing the plausibility of a Type II error in this study requires a more detailed analysis of the phenomenon of synchrony in female rats.

From the beginning, there has been no compelling theoretical reason to expect to find synchrony among female rats [19], nor has the phenomenon ever been replicated. A
test of the coupled-oscillator model of synchrony among Norway rats failed to confirm its basic assumptions [36]. Moreover, that study found an opposite effect; airborne odors from the ovulatory phase of the estrous cycle reduced cycle lengths rather than lengthening them [35]. This result may have been due to differences in apparatuses used in the first [20,23] and second studies [35,36], but similar cycle-reducing effects of ovulatory odors on female estrous cycles were found in yet a third apparatus [7]. These considerations alone suggest that a Type II error is not likely to explain the failure to find synchrony in this study.

Even more salient is methodological issues concerning the original study on estrous synchrony among rats [18]. That study consisted of two experiments, which used offspring derived from Charles River CD Sprague–Dawley rats and raised in all female groups of three to six rats. (The ages of the females at the time of the study were not reported.) In experiment I, 30 females exhibiting regular 4-day estrous cycles (estimated from 15 days of vaginal smears prior to the experimental conditions) were selected from these groups. Of these 30 females, 15 were initially housed in three groups of five females and the other 15 were housed individually and vaginal smears recorded for 30 days (Part I). The housing conditions were then reversed for these two sets of females and they were observed for another 30 days (Part II). Two control groups were formed for both parts of this experiment. The first set of control groups were constructed by randomly assigning the vaginal smear records of the 15 isolated females to three groups of five females, and the second set of control groups were constructed by randomly assigning the vaginal smear records of the 15 grouped (treatment) females to three groups of five females. McClintock [18] reported that the grouped females in both parts of the experiment differed from both types of random control groups ($P \leq .01$, the type of statistical analysis used was likely either the Mann–Whitney $U$ or $t$ test).

However, a cursory examination of Figs. 1 and 2 in Ref. [18] indicates that the main source of change in synchrony level was due to a decrease in synchrony of the “random” control groups with only a small increase in the synchrony of grouped (treatment) animals. Because the control groups were randomly constructed from the vaginal smear records of the experimental animals, the expectation is that on average they should not have changed in level of synchrony over time. Nevertheless, all four random control groups decreased in synchrony over time. This is a statistical artifact and may have been due to small sample size (i.e., only three groups for each condition).

In experiment II, 40 females exhibiting regular 4- or 5-day cycles were used, 20 in the spring and 20 in the fall [18]. Four air-recirculating units each housed five females in isolation. Thus, each group of five females shared a common recirculated air supply. Control groups were again randomly constructed from the vaginal smear records of the 20 rats used in each of the two phases of experiment II. In experiment II, although the level of synchrony was the same as in experiment I, we do not know whether the effect was due to synchronization of cycles in the treatment condition or to the decrease in synchrony of the random controls. Again, a likely statistical artifact of using small sample sizes.

Finally, of more general importance are the limits on synchrony imposed by cycle irregularity and the expected level of synchrony among individuals in a group. Both are also important methodological issues in the menstrual synchrony literature [1,31,32,38,60]. These topics are discussed in turn.

4. Cycle irregularity and the limits of synchrony

4.1. Methods

For a pair of rats, deviations in cycle length away from 4-day cycles should be a predictor of the maximum degree of synchrony attainable by the pair. There are at least two types of deviations away from 4-day cycles that may predict these limits. First, the rat in a pair with the maximum frequency of non-4-day cycles (i.e., an irregularly cycling rat) should be negatively correlated with the maximum degree of match between the pair. Second, the absolute difference in the frequency of 4-day cycles between a pair of rats should also be negatively correlated with the maximum degree of match (e.g., a frequent 4-day cycling rat paired with an irregular cycling rat should have a relatively low degree of match among cycle states). Since, both of these predictors should be negatively correlated with the maximum degree of match between animals, a positive predictor can be formulated by pooling these two predictors and subtracting them from 1:

$$X = 1 - \frac{1 - \min(f_1, f_2) + |f_1 - f_2|}{2}$$

(2)

where $X$ is the predicted maximum level of synchrony, $f_1$ and $f_2$ are the frequencies of 4-day cycles for two rats, “min” is the minimum frequency of 4-day cycles between the two, and the maximum frequency of non-4-day cycles is $1 - \min(f_1, f_2)$. The second term is the absolute value of the difference between the frequencies. For example, if two rats only have 4-day cycles, then $1 - \min(f_1, f_2)$ = 0 and $|f_1 - f_2|$ = 0, so the maximum degree of predicted match is 1. Eq. (2) is therefore a synchrony compatibility predictor for pairs of cycling females.

4.2. Results and discussion

Regression analysis revealed that Eq. (2) predicted the maximum possible match between estrous-cycle states: Match = 0.349 + 0.619X, $r^2 = .78$, $F(1, 9) = 27.6$, $P < .001$ (Fig. 5). The higher the frequency of 4-day cycles in both animals, the higher the maximum synchrony attainable by
the pair, and the lower the frequency of 4-day cycles in at least one animal, the lower the maximum synchrony. Eq. (2) also predicted the minimum mismatch between cycles as a function of regularity: \( \text{Match} = 0.1498 - 0.113X \), \( r^2 = 0.488 \), \( F(1, 9) = 7.6, P < .025 \) (Fig. 5b).

In Table 2, pairs 4 and 5 have the lowest synchrony compatibility, and they also have the lowest maximum Match. On the other hand, pairs 4 and 5 have the highest minimum mismatch as predicted by low values of Match. Thus, Eq. (2) predicts that for low synchrony compatibility, the range of possible matching and mismatching between a pair of females is very small and constrained to values expected by chance. Conversely, for high values of Match, the range approaches 0 to 1. Thus, cycle irregularity limits the degree of synchrony possible between females. A high degree of cycle irregularity, as operationally defined by Eq. (2), constrains the relationship of estrous-cycle states to chance levels of synchrony.

Fig. 6 illustrates the estrous-cycle distributions for this study, the group, and olfactory conditions for the previous estrous synchrony study (i.e., the cycle-length distribution for the grouped females in experiments I and II of Ref. [18] are depicted in Fig. 1 of Ref. [19] and reproduced as part of Fig. 6). As illustrated, the rats in the original study were at least if not more irregular than in this study. Extrapolating to groups of five, a high degree of match (i.e., synchrony) was not likely in the original study given the high level of cycle irregularity reported (see Fig. 6 and Fig. 1 in Ref. [19]).

### 5. Simulation: synchrony or asynchrony?

#### 5.1. Methods

In this study, vaginal smear classes were interpreted as estrous-cycle states (see Fig. 1). The original study [18], however, directly compared vaginal smear classes of females (i.e., L, LN, LC, N, NL, NC, C, CL, CN). Because the number of smear classes used was \( k = 9 \), a match of two out of five animals on any given day may indicate a small but significant degree of synchrony.

Classifying the synchrony level of females according to smear class is a combinatorial matching problem, but simpler than cycle-state matching used above because we are not concerned with the sequence of states, just with the match or mismatch in smear class states among animals on any given day. For example, suppose we observed 1000 groups of five female rats each for 30 days after they were grouped or regrouped. For group no. 1, we might observe \( \text{C, LN, N, LN, LC} \), and for group no. 531, we might observe \( \text{L, C, C, LN, C} \) on day 30. There are two animals that match in group no. 1 for the LN smear class and three animals that match in group no. 531 for the C smear class.
Thus, by sampling 1 day from each group, we can estimate the expected frequency of matches of smear classes that occur by chance.

The main complication in using computer simulation to estimate the expected frequency of synchrony by chance is determining the frequency of each smear class. When all smear classes are equally frequent (i.e., each occurs with probability 1/9), the expected degree of synchrony is again given by Eq. (1), for \( k = 9 \) (the number of smear classes). If all smear classes are equally frequent, then the variance in the smear-class frequency distribution is zero, but if all classes are not equally likely, then the variance must be greater than zero. There are many different frequency distributions with the same variance, but we can use Monte Carlo simulation to randomly sample from all possible frequency distributions to estimate how the level of synchrony systematically changes with variance in the frequency distributions of smear classes.

In this simulation, nine smear classes were distinguished (see Table 3). The greater the variance, the greater the expected synchrony (i.e., because with high variance, most individuals will tend to be in relatively few smear classes). Random numbers were used to generate frequency distributions of smear classes, and the variance was calculated as

\[
\sigma^2 = \frac{1}{9} \sum_{i=1}^{k=9} (x_i - 1/9)^2
\]

for each of 100 randomly generated frequency distributions with 1000 trials each. After 100 sets of 1000 trials were run, the mean degree of match was plotted against the variance for each set to yield a graphical relationship between smear class variance and synchrony.

Each trial consisted of assigning five model rats to a smear class based on the frequency of that class. For example, if LN occurs 30% of the time and L 3%, then a rat is 10 times more likely to be assigned to the LN smear class than the L class on any given trial. Once each smear class was assigned, the number of individuals with matching smear classes was calculated.

The procedure for calculating synchrony scores followed McClintock [18]. No matches were assigned a score of 0 (e.g., L, LN C, N, LC). Exactly two, three, four, and five matches were assigned the same score as the number of matches (e.g., a synchrony score of 5 might be LN, LN, LN, LN, LN or C, C, C, C, C). There were two special cases. First, when two pairs of smears matched (e.g., LN, N, C, N, LN), then a synchrony score of 3 was assigned to the group. Second, if three and two smears matched (e.g., C, LN, C, C, LN), then a synchrony score of 4 was assigned.

5.2. Results and discussion

The relationship between smear-class variance and synchrony has a logistic-like shape (Fig. 7). Synchrony is at a minimum when all smear classes occur with equal frequency. The mean score can be calculated exactly using Eq. (2) and estimated by this simulation technique. In both cases, the expected value is 1.74, which is the minimum synchrony expected for five individuals and nine equally frequent smear classes. As the variance increases, so do the average synchrony scores. Using the smear class frequencies from this study (Table 3), the expected degree of synchrony would be about 2.8. The scores of 2.14 and 2.28 reported in Ref. [18] are also plotted in Fig. 7 in three ways. They are plotted as (i) “solid diamonds” assuming no variance (they are slightly above the expected synchrony level), (ii) as “open circles” assuming a low level of variance that corresponds to the simulation expectation for those scores, and (iii) as “solid squares” plotted against the same level of smear-class variance as the data presented here indicating a lower than expected degree of synchrony (Table 3).

If all smear classes occur with equal frequency, then the expected degree of match is just 1.74. The actual frequency distribution of smear classes was not reported [18], but the cycle-length distributions were subsequently reported [19].

![Fig. 7. Plot of 100 mean synchrony scores (1000 trials each) for groups of five simulated animals with different degrees of variance in the smear-class frequency distributions. McClintock’s [18] synchrony scores are plotted three ways assuming: (i) no variance in smear classes ( ); (ii) variance two to three times less than the data reported in this study, and based the variance required to fit these points to the theoretical curve of this simulation ( ); and (iii) with the same variance as reported in this study ( ), which generated an expected synchrony level of 2.8.](image-url)
(see Fig. 6). The frequencies of smear classes could not have been the same for all classes, because non-4-day cycles will have sequential L smears, C- or N-dominated smears or both (e.g., see Fig. 1). Fig. 6 indicates that cycle-length irregularity was at least the same or greater in the original study than here. It is possible that the smear class variation in the original study was less than in this study because sequences of persistent estrous smears were excluded from the original study. However, this would not eliminate smear-class variance. Therefore, mean synchrony scores in the range of 2.14 and 2.28 would be statistically consistent with chance levels of synchrony even if there was little variation in the frequency of smear classes (Fig. 7).

6. General discussion

This study failed to detect synchrony among pairs of sibling females that were housed together. There are several possible explanations for the failure to detect synchrony including the possibility of a Type II error. To assess the plausibility of a Type II error, further analysis of the phenomenon of estrous synchrony was performed. Because estrous synchrony among female Norway rats has been reported in only one other study [18], that study and the present study were necessarily the focus of this analysis.

The analysis revealed two general points. First, persistent cycle variability greatly restricts the degree of synchronizaton possible between rhythms. The problem of cycle variability, especially between-female cycle variability has been recently recognized in menstrual synchrony research to be a mathematical obstacle to synchrony [1,3,12,38,39,60]. In Fig. 5, we see that the maximum synchrony attainable by a pair of female rats is increasingly constrained as cycle variability increases. We also see in Fig. 6 that the cycle variability in the original study was at least as great if not greater than in this study. Thus, cycle variability is a fundamental constraint on the maximum degree of synchrony attainable among females. This is of ecological significance because the estrous cycles of wild Norway rats are likely longer and more irregular than domestic strains [2,15], suggesting that the maximum degree of synchrony in wild populations is even more constrained than in domestic. Second, the computer simulation analysis of the expected degree of synchrony in groups of five rats based on their vaginal smear types revealed that unless females are very regular cyclers, synchrony scores of 2.14 and 2.28 are consistent with chance levels.

How could significant levels of synchrony have been found in the original study? There are two mutually reinforcing errors that may have contributed to Type I errors. First, the effect of synchrony in the original study was mainly due to a decrease in synchrony of the randomly constructed control groups. Randomly constructed control groups should, on average, neither increase nor decrease over time. The decrease observed was likely due to small sample size, and thus a statistical artifact. Second, the decrease in synchrony level of the control groups was amplified by treating the individual rather than the group as the unit of analysis. Synchrony is a relationship among the individuals in a group and not a characteristic of an individual [29]. Because each individual in a group of five received the same score, the degrees of freedom were inflated by approximately a factor of 5 and thereby increasing the likelihood of a Type I error.

Perhaps by focusing on synchrony, researchers have overlooked the reproductive costs of synchrony to females. Avoiding synchrony may reduce inter-female competition for males [8,27]. Avoiding synchrony may also reduce the predictability of future female ovulations and perhaps reduce the likelihood that a male can control a female’s matings by predicting her next ovulation [25]. Coupled-oscillator mechanisms can generate cycle irregularity, synchrony and asynchrony [34], suggesting that the avoidance of synchrony may be selected for. However, it should be emphasized that the coupled-oscillator mechanism is not required to avoid synchrony. Avoidance of synchrony, as we have seen, is a mathematical property of cycle irregularity (Fig. 5). Thus, the avoidance of synchrony need not have been selected for to enhance female choice. Instead, the avoidance of synchrony — as a product of irregular cycles — may be an intrinsic property of the physiological processes that generate ovarian cycles in mammals.

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