Reciprocal inheritance of centrosomes in the parthenogenetic Hymenopteran *Nasonia vitripennis*

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Background: In the majority of animals, the centrosome – the microtubule-organizing center of the cell – is assembled from components of both the sperm and the egg. How the males of the insect order Hymenoptera acquire centrosomes is a mystery, as they originate from virgin birth.

Results: To address this issue, we observed centrosome, spindle and nuclear behavior in real time during early development in the parthenogenetic hymenopteran *Nasonia vitripennis*. Female meiosis was identical in unfertilized eggs. Centrosomes were assembled before the first mitotic division but were inherited differently in unfertilized and fertilized eggs. In both, large numbers of asters appeared at the cortex of the egg after completion of meiosis. In unfertilized eggs, the asters migrated inwards and two of them became stably associated with the female pronucleus and the remaining cytoplasmic asters rapidly disappeared. In fertilized eggs, the *Nasonia* sperm brought in paternally derived centrosomes, similar to *Drosophila melanogaster*. At pronuclear fusion, the diploid zygotic nucleus was associated only with paternally derived centrosomes. None of the cytoplasmic asters associated with the zygotic nucleus and, as in unfertilized eggs, they rapidly degenerated.

Conclusions: Selection and migration of the female pronucleus is independent of the sperm and its aster. Unfertilized male eggs inherit maternal centrosomes whereas fertilized female eggs inherit paternal centrosomes. This is the first system described in which centrosomes are reciprocally inherited. The results suggest the existence of a previously undescribed mechanism for regulating centrosome number in the early embryo.

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Background

The centrosome is the primary microtubule-organizing center of the animal cell (reviewed in [1,2]). It nucleates microtubule assembly and determines the number, length and overall distribution of microtubules within the cell. Thus, the centrosome plays a key role in maintaining cytoarchitecture and the cellular distribution of the nucleus and other organelles. Animal centrosomes typically contain a perpendicularly oriented pair of microtubule-based centrioles surrounded by a protein-rich, electron-dense cloud of pericentriolar material.

In the vast majority of animals, the centrosome is disassembled during gametogenesis in males and females and then reassembled soon after fertilization, requiring components from both sperm and egg ([3], reviewed in [4–6]). During spermatogenesis, the centrosome is stripped of the pericentriolar material and its ability to nucleate microtubules, but retains the centriole and its ability to replicate. Conversely, during oogenesis, the centrosome loses its ability to replicate but retains the pericentriolar material, whose components become dispersed throughout the cytoplasm [4]. Thus, zygotic centrosome assembly at fertilization utilizes the sperm's centriole and the egg's pericentriolar material (reviewed in [2,4,6]). In *Drosophila melanogaster*, for example, the sperm basal body is a single centriole that disassociates from the sperm immediately after fertilization and becomes associated with maternally supplied centrosomal proteins ([7,8], reviewed in [9]).

In *Drosophila* and other insects, the sperm-derived centriole serves two functions during early development: nucleating zygotic centrosome formation, and selection of the female pronucleus from among the four female meiotic products in the egg (reviewed in [2,6,10]). As the *Drosophila* oocyte completes meiosis, the newly assembled centrosome duplicates and nucleates two distinct foci of microtubule arrays. These arrays interact with the innermost of the four female meiotic products, which becomes the female pronucleus. The microtubule arrays appear to serve as tracks for migration of the female and male pronuclei towards each other.

While biparental reproduction is the norm, development in the absence of fertilization is not uncommon among animals, especially insects (reviewed in [11,12]). The

insect order Hymenoptera (which includes ants, bees and wasps), for example, is characterized by a mode of parthenogenesis called haplodiploidy, whereby unfertilized eggs develop into males and fertilized eggs develop into females. Parthenogenetic animals provide a unique opportunity for investigating alternative strategies of centrosome inheritance. The recent report of microtubule organization in parthenogenetic eggs of *Muscidifurax uniraptor* [13] is one of very few studies that have exploited this opportunity. In unfertilized *M. uniraptor* eggs, a large number of microtubule-based astral arrays are present in the cytoplasm during meiosis and the first three mitotic divisions [13]. These arrays contain centrioles and numerous centrosomal components, including gamma-tubulin, clearly demonstrating that maternal components alone can assemble centrosomes during parthenogenesis [13]. However, because of their transient nature, it is difficult to determine the function of these asters and to establish definitively their relationship to the zygotic centrosome by analyzing fixed embryos.

We have therefore developed techniques to follow astral dynamics in living hymenopteran embryos. We chose to analyze embryos from the hymenopteran *Nasonia vitripennis* because it oviposits multiple eggs per host and its biology has been well characterized [14]. Here, we report that female pronuclear selection does not depend on a perm-derived aster, and that centrosome inheritance occurs reciprocally in *Nasonia*. Unfertilized male eggs inherit maternal centrosomes and fertilized female eggs inherit paternal centrosomes.

Results

Female pronuclear selection occurs independently of the sperm and its aster

The principal aster present in the majority of fertilized eggs is sperm derived [4]. It plays a critical role in mediating the migration of the male and female pronuclei towards each other [4], and it is thought to be important for selecting the female pronucleus from among the four meiotic products in animals in which all four remain in the egg, as occurs in *D. melanogaster* [10]. In *Nasonia*, all four meiotic products also remain in the egg and, thus, all could potentially become the female pronucleus [15,16] (Figure 1d,h,l). Given these observations, an alternative mechanism must be used to specify the female pronucleus in the absence of a sperm-derived aster in unfertilized *Nasonia* embryos. To address this issue, we analyzed microtubule and chromosome dynamics during meiosis in unfertilized and fertilized *Nasonia* eggs. To analyze microtubule and nuclear dynamics simultaneously in living embryos, we microinjected a cocktail of rhodamine-labeled tubulin and the DNA marker OliGreen into freshly laid fertilized and unfertilized embryos, and followed them using time-lapse laser-scanning confocal microscopy.

Meiosis II in unfertilized (Figure 1a–d) and fertilized (Figure 1e–h) eggs appeared identical. As in *Drosophila*, the spindles were localized anteriorly at the cortex [17,18]. These spindles were anastral and separated by a robust spherical tubulin structure or 'middle-pole material' that lies between the chromosomes [19,20] (Figure 1a–k, arrowhead). During anaphase/telophase, the innermost nucleus migrated away from the other three meiotic products (Figure 1c,g). In all cases ($n = 7$, unfertilized; $n = 4$, fertilized), this innermost migrating nucleus became the female pronucleus (Figure 1d,h,l, arrow) while the polar bodies and the middle-pole material remained in the cortical region (Figure 1j,k). Because meiosis appears identical in fertilized and unfertilized eggs, this suggests that the sperm does not play a critical role in female pronuclear selection and migration. Instead, it appears that the middle-pole material may play a crucial role. Mutations that disrupt the middle-pole material in *Drosophila* eggs result in defects in meiosis and/or pronuclear migration and fusion [21,22]. In *Nasonia* embryos, the middle-pole material may function in female pronuclear selection by acting as a tether to prevent the other three meiotic products from migrating into the interior of the egg, where the mitotic divisions are initiated.

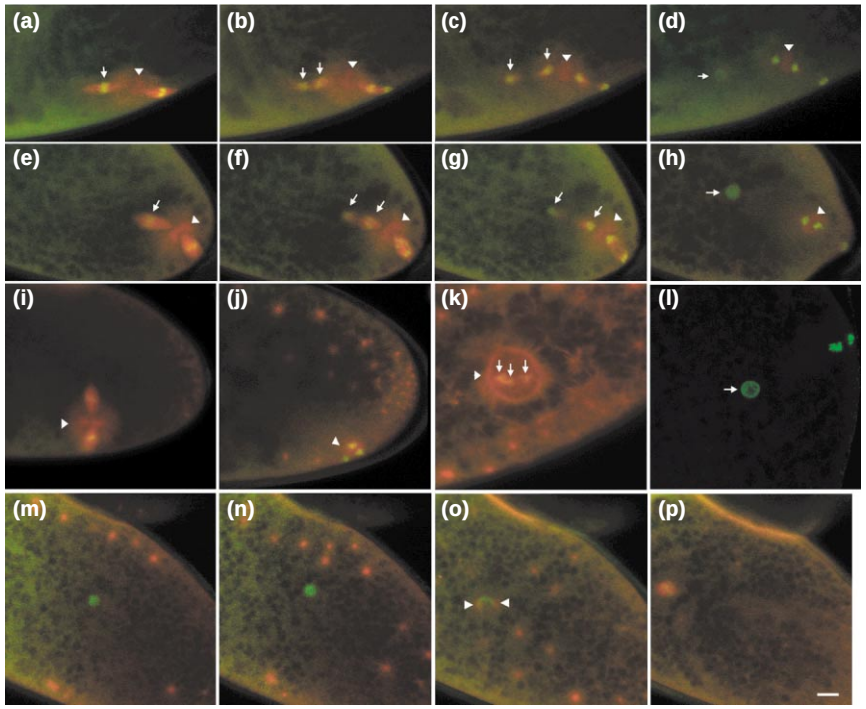
Unfertilized embryos inherit centrosomes maternally

After the completion of meiosis, numerous cytoplasmic microtubule asters appeared along the cortex in both unfertilized and fertilized *Nasonia* eggs (compare Figure 1i,j, which show during and after meiosis II, respectively). These asters were present for approximately 30 minutes and were no longer detectable by the end of the first mitotic division (Figure 1p). Although we have not counted them, our observations are consistent with Riparbelli *et al.*'s finding that they number in the hundreds in *M. uniraptor* eggs [13]. Shortly after their appearance at the cortex in *Nasonia* eggs, the asters migrated from the cortex towards the interior of the egg (Figure 1m–o). In unfertilized embryos, two of the cytoplasmic asters became associated with the female pronucleus (Figure 2). The female pronucleus acquired the asters one at a time, in quick succession, capturing the second aster within minutes of the first one (Figure 2a–h). Although numerous cytoplasmic asters were in close proximity to the female pronucleus, it never captured more than two. The two captured asters became the zygotic centrosomes and organized the first mitotic spindle (Figure 2i). The remaining cytoplasmic asters disappeared by the end of the first mitotic division (Figure 2i–l). These observations indicate that unfertilized embryos inherit two centrosomes from a maternal pool of cytoplasmic asters.

Fertilized embryos inherit centrosomes paternally

To determine whether fertilized embryos inherit their centrosomes from the egg cytoplasm or from the fertilizing sperm, we analyzed microtubule and chromosome dynamics

Figure 1



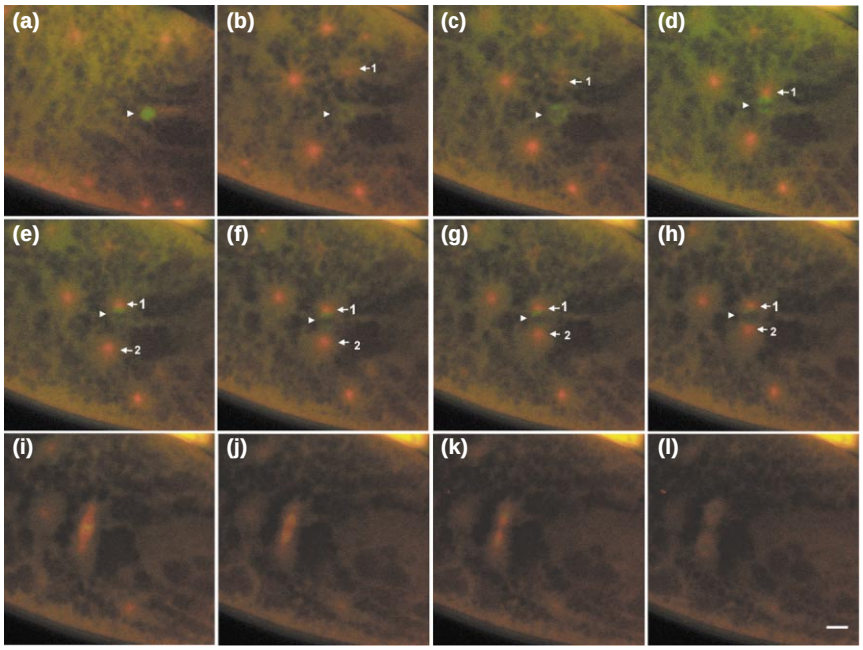
Meiosis in (a–d) unfertilized (n = 7) and (e–h) fertilized eggs (n = 4) appear identical. Microtubules are labeled red and DNA green. (a,e) Metaphase II was the earliest event observed. (d,h) The innermost meiotic product migrated to the interior and became the female pronucleus (arrow). Analysis of (l) unfertilized and fertilized (not shown) embryos fixed and stained for DNA confirmed that the innermost product always became the female pronucleus. (i–k) The middle-pole material (arrowhead, also indicated in (a–h)) appeared to tether the other three meiotic products (arrows in (k)), preventing them from migrating deeper into the egg interior. Numerous cytoplasmic asters appeared at the cortex of both fertilized (not shown) and unfertilized eggs at the end of meiosis II (compare (i,j), which show meiosis II and the end of meiosis, respectively). (m–p) In unfertilized eggs, the asters migrated towards the female pronucleus and two associated with it (arrowheads in (o)) while the remainder disappeared by the end of the first mitotic division (p). The scale bar represents 20 μm.

in fertilized eggs. Our analysis of live embryos confirms previous studies indicating that sperm entry occurs while the oocyte is completing meiosis [15,16]. We found that, immediately following entry, the sperm was initially rod shaped with two closely associated asters (Figure 3a). It is very likely that these microtubule asters are organized by paternally derived centrioles as sperm entry occurs during early meiosis and the maternal cytoplasmic asters have not yet formed in the oocyte (Figure 3a–d). After sperm entry, the rod-shaped sperm nucleus became spherical in its transformation into a male pronucleus (Figure 3a–d). At this point, the male pronucleus possessed two paternally derived centrosomes, and maternally derived cytoplasmic asters began forming along the cortex (Figure 3e). The female pronucleus (arrowhead) migrated towards the male pronucleus (arrow), but did not acquire any cytoplasmic asters during its migration (Figure 3f–h). As the female pronucleus migrated towards the male pronucleus, the cytoplasmic asters did not display much movement towards the interior of the egg (compare Figure 3e,f). When the two pronuclei fused, the product contained only the paternally derived asters (Figure 3h, inset, open red arrowheads). By this time, the cytoplasmic asters had begun to migrate towards the interior of the egg (Figure 3i). Although cytoplasmic asters were present and migrated towards the interior as they did in unfertilized eggs, none of them became associated with the fused pronuclei. The paternally derived centrosomes organized the first mitotic spindle and, as in unfertilized embryos, the maternally derived cytoplasmic asters disappeared by the end of the first mitotic division (Figure 3i–l). Thus, unlike unfertilized eggs, fertilized *Nasonia* eggs inherit their centrosomes paternally.

Discussion

We have shown that female meiosis in *Nasonia* is identical whether or not the egg has been fertilized, indicating that the sperm does not play a critical role in selection of the female pronucleus. In *Drosophila*, specification of the innermost of the four meiotic products as the female pronucleus is thought to rely on interactions with the sperm aster [10]. It is postulated that the female pronucleus is transported towards the male pronucleus along the sperm aster by a minus-end-directed motor protein. In *Nasonia*, we observed that the innermost meiotic product migrated towards the interior of the egg while the polar bodies remained near the cortex, embedded in the middle-pole material, in both unfertilized and fertilized eggs. Migration of the female pronucleus is therefore not dependent on the microtubule asters emanating from the sperm in *Nasonia* eggs. This observation together with mutational analysis of the *Drosophila klp3A* gene suggests a different model for female pronuclear specification/selection. In eggs deficient for KLP3A, a plus-end directed kinesin-like protein, meiosis is normal despite the fact the middle-pole material is diminished, and the female pronucleus does not migrate towards the male pronucleus even

Figure 2



Unfertilized embryos acquire their centrosomes from the cytoplasmic pool of maternal asters (n = 8). Microtubules are labeled red and DNA green. **(a)** Centrosomes were initially found at the cortex and the female pronucleus (arrowhead) in the interior of the egg. **(b,c)** Within minutes, the centrosomes began their migration inwards. The female pronucleus captured **(d)** the first centrosome (arrow 1) and **(e–h)** the second centrosome (arrow 2) within minutes of each other. **(i)** The captured centrosomes organized the first mitotic spindle. **(j–l)** Additional centrosomes were not captured and disappeared by the end of the first mitosis. The scale bar represents 20 μm.

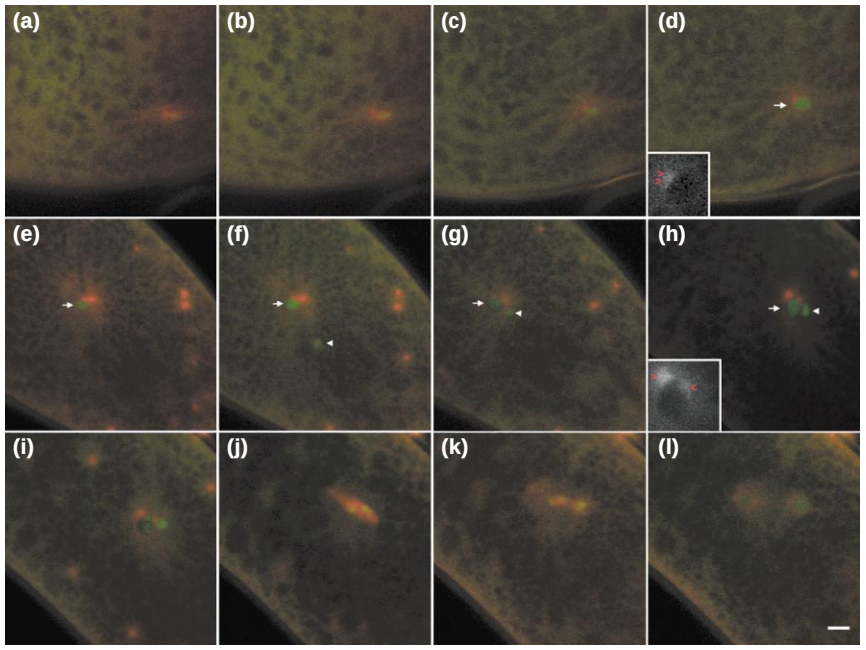
though it nucleates a normal aster [21]. Williams *et al.* [21] proposed that KLP3A might function at the middle-pole material as a 'pushing force' that propels the female pronucleus towards the interior, a model supported by our real-time analysis of meiosis in *Nasonia*.

We have found that the *Nasonia* egg assembles numerous microtubule-based asters after egg activation/completion of meiosis. While tubulin clearly marks the microtubule-organizing center, it will be important to determine whether they contain centrioles and core centrosomal components such as gamma-tubulin. Riparbelli *et al.* [13] have shown that gamma-tubulin and centrioles are indeed present in cytoplasmic asters in unfertilized eggs of the parasitoid wasp *M. uniraptor*. That the asters in *Nasonia* eggs assemble specifically at the cortex suggests that gamma-tubulin and other centrosomal material may be specifically localized at the cortex [23]. It is not known what triggers asters to form in *Nasonia* eggs. It has been demonstrated, however, that an increase in cytoplasmic pH and calcium ion levels are sufficient for stimulating aster formation in artificially activated sea urchin eggs [24,25]. These changes, which also occur during natural egg activation and fertilization, are hypothesized to regulate the level of tubulin present in the egg, which influences their assembly into asters [24,25]. We found the rate of aster movement in *Nasonia* eggs to average 0.09 ± 0.01 μm/second from the cortex towards the egg interior, suggesting that it is mediated by microtubule-based molecular motors [26].

Regulatory mechanisms that prevent an inappropriate accumulation of centrosomes are critical for proper development as supernumerary centrosomes are potentially destructive, organizing multipolar spindles that lead to unequal chromosome segregation and abortive development [27]. These mechanisms, however, remain largely uncharacterized. In organisms in which the centrosome is derived from the sperm basal body, loss or inactivation of the maternally derived oocyte centrosomes maintains proper centrosome number. In the surf clam *Spisula solidissima*, for example, differential regulation of maternal and paternal centrosome activity prevents the multiple centrosomes present in the egg from organizing abnormal spindles [28]. Fertilization occurs during prophase of meiosis I resulting in the presence of three active centrosomes, two maternally derived and one paternally derived [28]. Wu and Palazzo have suggested that during meiosis I, the microtubule nucleating activity of the paternal centrosome is specifically inhibited. By the time the nucleating activity of the paternal centrosome is restored during meiosis II, the maternal centrosome has been reduced to 'one-half centrosome' which is not capable of reproducing itself. Wu and Palazzo suggest that a combination of specific inhibition of the paternal centrosome and reduction of the maternal centrosome during meiosis serve as the reductional mechanism to achieve appropriate centrosome number.

From our analysis of *Nasonia*, we present an alternative model in which the appropriate centrosome number is

Figure 3



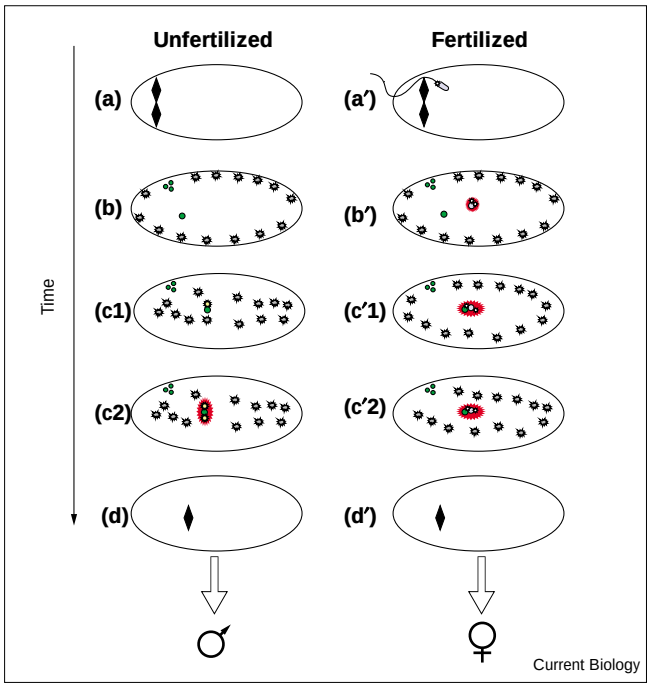
Fertilized embryos inherit their centrosomes from the fertilizing sperm (n = 10). Microtubules are labeled red and DNA green. The sperm entered the egg during the early stages of female meiosis and before the appearance of cytoplasmic asters. **(a–d)** The sperm was initially rod shaped and had two centrosomes associated with it, but then transformed to form a spherical male pronucleus (arrow) and had two paternally derived centrosomes. The inset in (d) shows the tubulin fluorescence only (red open arrowheads denote the two centrosomes). **(e–g)** By this time, cytoplasmic asters were present at the cortex. The female pronucleus (arrowhead) migrated towards the sperm pronucleus (arrow). The female pronucleus did not capture any of the cytoplasmic centrosomes before its association with the male pronucleus. **(h,i)** Migration of the cytoplasmic asters towards the interior of the egg became apparent after the pronuclei had become apposed (compare with (f,g)) The inset in (h) shows the tubulin fluorescence only (red open arrowheads denote the two centrosomes). **(j,k)** The paternally derived centrosomes set up the first mitotic spindle. **(l)** Cytoplasmic centrosomes did not become associated with the pronuclei pair and disappeared by the end of the first mitotic division. (j,k) As previously reported, the first division was gonomeric, the maternal and paternal chromosome sets remained separate until telophase [16]. The scale bar represents 20 μm.

achieved by a process in which centrosomes associated with the nuclear envelope become selectively stabilized and that once a nucleus is associated with two centrosomes, regardless of their origin, a mechanism prevents it from associating with more. We observed that, by the end of the first mitotic division, only asters associated with nuclei were visible and free asters had disappeared. It is possible that free asters are labile and that nuclear association stabilizes them, through interactions with the nuclear envelope, chromatin and/or kinetochore [13,29]. Once two centrosomes are associated with a nucleus, we hypothesize that they nucleate sufficient astral microtubules to repel additional centrosome attachment. Together, these mechanisms ensure that the zygotic *Nasonia* nucleus becomes endowed with the correct number of centrosomes.

These mechanisms regulating centrosome number are not likely to be specific to *Nasonia* embryos. In *Sciara coprophila*, the sperm basal body contributes 6–10 microtubule-organizing centers at fertilization and yet each zygotic nucleus does not possess more than two [30]. In newt eggs, which are regularly fertilized by multiple sperm, only one sperm fuses with the female pronucleus to form the zygote [31]. Once this occurs, the remaining sperm and their asters degenerate [31]. Whether similar mechanisms operate in somatic cells is not known. Checkpoints are required to prevent abnormal centrosome amplification in somatic cells [32]. Monitoring the number of centrosomes associated with the nuclear envelope may represent an additional mechanism by which centrosome number is regulated.

Our analysis of *N. vitripennis* represents the first example of reciprocal centrosome inheritance: unfertilized male embryos inherit their centrosomes maternally and fertilized female embryos inherit their centrosomes paternally. Centrosome inheritance is paternal in the vast majority of animals [4]. Rodents exhibit an exceptional maternal centrosome inheritance pattern [33–35]. In unfertilized *Nasonia* eggs, the only source of centrosomes is the maternal pool. Though centrosomes are available from both the maternal cytoplasm and the sperm in fertilized *Nasonia* eggs, it is the paternally derived centrosomes that organize the first mitotic division. Our observations suggest that this is a direct result of the fact that pronuclear fusion occurs prior to the migration of cytoplasmic asters (Figure 4). Migration of the female pronucleus towards the male pronucleus occurs while the cytoplasmic asters are relatively stationary at the cortex (see Figure 3). The female pronucleus reaches the male pronucleus, which has two associated centrosomes, prior to or concomitant with the migration of the cytoplasmic asters. As discussed above, the fused pronuclei with their associated paternal centrosomes do not associate with the migrating maternal centrosomes.

Figure 4



Model for reciprocal centrosome inheritance in *Nasonia*. The *Nasonia* egg completes meiosis after egg deposition and appears identical in unfertilized and fertilized eggs. **(a,a′)** The earliest stage examined was metaphase II (t = 0). **(b,b′)** At the end of meiosis, numerous cytoplasmic asters (gray starbursts) appear at the cortex ((b), t = 19.2 ± 2.2 min; (b′), t = 19.3 ± 3.9 min). **(c1,c2)** In unfertilized eggs, the asters migrate inward and two are captured (yellow starbursts) by the female pronucleus (green circle; (c1), t = 26.2 ± 2.6 min; (c2), t = 30.5 ± 2.1 min). (c2) Once the female pronucleus is associated with two asters, a mechanism (red starburst encompassing the nucleus and its two centrosomes) prevents more asters from associating with it. (a′) In fertilized eggs, the sperm (blue oval with tail) brings in two paternally derived centrosomes (white starbursts; t = 0). (c′) The female pronucleus reaches the male pronucleus (blue circle) before the migration of the cytoplasmic asters and does not acquire any cytoplasmic asters (t = 29.8 min). (b′,c′) As the male pronucleus already has two centrosomes, additional asters are prevented from associating with it and with the pronuclei pair. **(d,d′)** The unassociated cytoplasmic asters disappear in both unfertilized and fertilized eggs before the end of the first division ((d), t = 41.9 ± 5.3 min; (d′), t = 45.9 min ± 7.6 min).

The significance of reciprocal centrosome inheritance is not known. One possibility is that it may function as a mechanism for maintaining the integrity of maternally inherited centrosomes. As unfertilized eggs inherit their centrosomes maternally and develop into males, the maternal centrosomes must be able to function both as a microtubule-organizing center during male meiotic and mitotic divisions as well as a basal body, which organizes the sperm axoneme. If the centrosomes inherited are defective, motile sperm may not be produced, rendering the male infertile, and thus effectively eliminating the defective centrosomes from the reproductive pool. If the centrosomes are functional, sperm will be produced and the male will be fertile and transmit functional centrosomes. These male centrosomes are then transmitted to fertilized eggs, which develop into females. Observations in *Drosophila* indicate that centrosome function at the mitotic spindle pole is separable from its function as a male meiotic spindle pole and basal body. For example, males carrying a mutation in the gene *mfs*, which encodes the *Drosophila* centrosome protein centrosomin, survive to adulthood but are sterile because they do not produce motile sperm [36]. Although the mitotic divisions appear normal in testes from *mfs* mutants, meiotic spindle organization is abnormal and the sperm axoneme is disorganized [36]. Thus, centrosomes that can drive mitotic divisions during somatic development may not necessarily be functional in directing meiotic divisions and spermatogenesis. Therefore, reciprocal centrosome inheritance in *Nasonia* may serve as a selective mechanism for maintaining the functional integrity of centrosomes. There are possibly multiple forms of centrosomes segregating in *Nasonia* populations, and reciprocal inheritance serves as a mechanism that selects and maintains those forms that are functional in a wide array of cell types.

Conclusions

Our real-time analysis of centrosome, spindle and chromosome behavior in live embryos from the parthenogenetic hymenopteran *N. vitripennis* have revealed that centrosomes are reciprocally inherited: unfertilized male embryos inherit maternally derived centrosomes, whereas fertilized female embryos inherit paternally derived centrosomes. This mode of centrosome inheritance relies on a previously undescribed mechanism, which selectively stabilizes centrosomes associated with the zygotic nuclei and which prevents a nucleus from associating with more than two centrosomes. This analysis also demonstrates that selection and migration of the female pronucleus does not rely on the presence of the sperm and its aster.

Materials and methods

Stocks

N. vitripennis wild-type strain AsymC was provided by John Werren (University of Rochester) and was maintained under constant light at 28°C. *Nasonia* are solitary parasitoids [14] that oviposit their eggs in cyclorrhaphous fly pupae, especially those of blowflies. Embryonic development lasts approximately 9–10 h and development from egg to adult takes approximately 11 days at 28°C [16]. *Sarcophaga bullata* pupae (Carolina Biological) served as the host species and as the oviposition substrate for egg collections.

Egg collection

Eggs were collected from unmated or mated females as described in Pultz *et al.* [37] with minor modifications. Females were at least 3–4 days old and were fed 10% sucrose for 2–3 days and then *Sarcophaga* overnight the night before egg collection. Females were set singly and allowed to oviposit for 30–45 min.

Microinjection of fluorescent probes

Collected eggs were aligned on a coverslip, desiccated 30–40 min under vacuum, and injected under halocarbon oil with a cocktail of OliGreen (Molecular Probes) and rhodamine–tubulin [38]. The OliGreen was first diluted threefold in PEM80 (80 mM PIPES, pH 6.8,

1 mM $MgCl_2$, 1 mM EGTA) and then was combined 1:1 with rhodamine–tubulin (12.9 mg/ml). The cocktail was injected at about 70% egg length (0, posterior end; 100, anterior end) according to standard *Drosophila* procedures [39].

Microscopy

Analysis was performed using an inverted microscope (Leitz DM IRB, Leica) equipped with a laser confocal imaging system (Leica TCS NT). Embryos were typically filmed for 30–60 min, with images taken every 30 sec or 60 sec, using the time-lapse feature of the TCS NT software.

Supplementary material

Movies accompanying Figures 1–3 are available at http://current-biology.com/supmat/supmatin.htm.

Acknowledgements

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