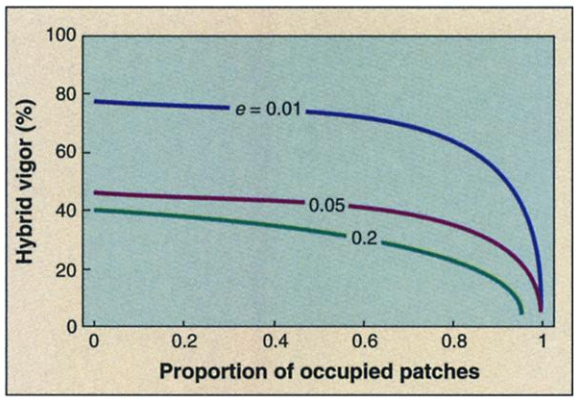
residents and immigrants, ruling out any influence of interactions between genotypes and the local environment of the rock pools in explaining hybrid success. The remaining hypothesis is that the observed hybrid vigor was a direct result of their escape from inbreeding depression. This confirms that inbreeding depression can be large in natural populations.

The strength of inbreeding depression reveals a puzzle. With fitness costs so high, why hasn't natural selection purged the numerous deleterious alleles that cause inbreeding depression? The answer probably lies in the metapopulation structure of *Daphnia*. A metapopulation is a population of populations: The individuals in the same rock pool constitute a subpopulation, and all rock pools constitute a metapopulation of subpopulations linked by dispersal. If dispersal is low, then subpopulations remain genetically distinct, and weakly selected deleterious alleles can reach high frequencies in local populations (*4, 5*). The existence of these weakly selected alleles sets the stage for inbreeding depression. The semi-isolation of subpopulations means that they are likely to differ with respect to the deleterious alleles they harbor. Therefore, benefits accrue among the hybrid offspring of residents and immigrants, because the bad effects of any (partly) recessive alleles they receive from one parent are likely to be masked by the alleles from the other parent.

How common are metapopulations and the potential for strong inbreeding depression in other species? One of the hallmarks of metapopulations is the appearance and disappearance of subpopulations from habitat patches (for example, rock pools) as a result of frequent extinction and recolonization. Ebert *et al.* report that *Daphnia* subpopulations in rock pools have a 20% chance of going extinct each year, and because dispersal (recolonization) is low, only 20% of the suitable rock pools are occupied in any given year. Thus, the metapopulation structure of *Daphnia* affects not only its genetic properties but also its demography, as measured by its presence or absence from rock pools.

To compare the effects of metapopulation structure on inbreeding and demography, we calculated the expected hybrid vigor (measured as the percentage gain in relative fitness of individuals whose parents come from different subpopulations compared to individuals produced from parents in the same subpopulation) for a hypothetical species sharing many of the population characteristics of *Daphnia*. In the figure, hybrid vigor is plotted against the expected proportion of suitable habitat patches occupied by the species, which is determined by the rates of subpopulation extinction and recolonization. The key point of the figure is that, as the number of occupied patches increases, hybrid vigor remains largely unchanged until almost no empty patches remain. This means that the genetic importance of metapopulation structure occurs even when recolonization rates are sufficiently high that the metapopulation structure is not apparent from the demography. The explanation for this is simple. From a demographic perspective, only a single disperser is needed to colonize a habitat patch, whereas many dispersers are needed to homogenize the genetics of distinct subpopulations.



A matter of inbreeding. Hybrid vigor versus proportion of occupied habitat patches for three different subpopulation extinction rates (e = probability of extinction per sexual generation). We assume that hybrid vigor is caused by many genes with mildly deleterious, partly recessive alleles (*7*). Migration of individuals into subpopulations and colonization of empty patches are assumed to be governed by the same processes. Although details of the curves depend on the particular assumptions we used in the calculations, the general patterns do not.

These results suggest that "population genetic metapopulations" and the concomitant potential for inbreeding depression may be quite common—perhaps more common than "ecological metapopulations" whose demographies are dominated by extinction and recolonization. Ecological metapopulations might be quite easy to spot in nature; the genetic effects of spatial population structure are likely to be more cryptic. Ebert *et al.* found high inbreeding depression in a species that has a clear ecological metapopulation structure (*6*), yet it is likely that the same processes that lead to inbreeding depression occur for species that less obviously live in metapopulations.

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PERSPECTIVES: CELL CYCLE

A Trigger for Centrosome Duplication

Yannick Arlot-Bonnemains and Claude Prigent

The centrosome is the cell's principal organizing center for microtubule assembly. Like DNA, the centrosome must be duplicated once and only once during each cell cycle. Centrosome duplication is important for assembly of the bipolar microtubule spindle to which replicated chromosomes are attached prior to cell division (mitosis). In the absence of centrosomes, the spindle appears to be assembled (*1*) but cells cannot undergo cleavage (cytokinesis) (*2*). During normal mitosis, each daughter cell inherits only one centrosome, thus ensuring that the correct number of centrosomes will be available for the next round of cell division. One intriguing question that still plagues the "centrosome community" is how centrosome duplication is regulated. On page 499 of this issue, Matsumoto and Maller report that a surge of calcium ions followed by activation of calmodulin- dependent kinase II (CaMKII) is the trigger for centrosome duplication (*3*).

Several years ago, it was discovered that activation of the cyclin E–Cdk2 (cyclin-dependent kinase 2) complex at the G_1-S phase transition (restriction point) of the cell cycle allowed both DNA replication and centrosome duplication to proceed (*4, 5*). The activated cyclin E–Cdk2 complex together with the Rb tumor suppressor protein enables cells to move from G_1 into S phase. When phosphorylated, Rb

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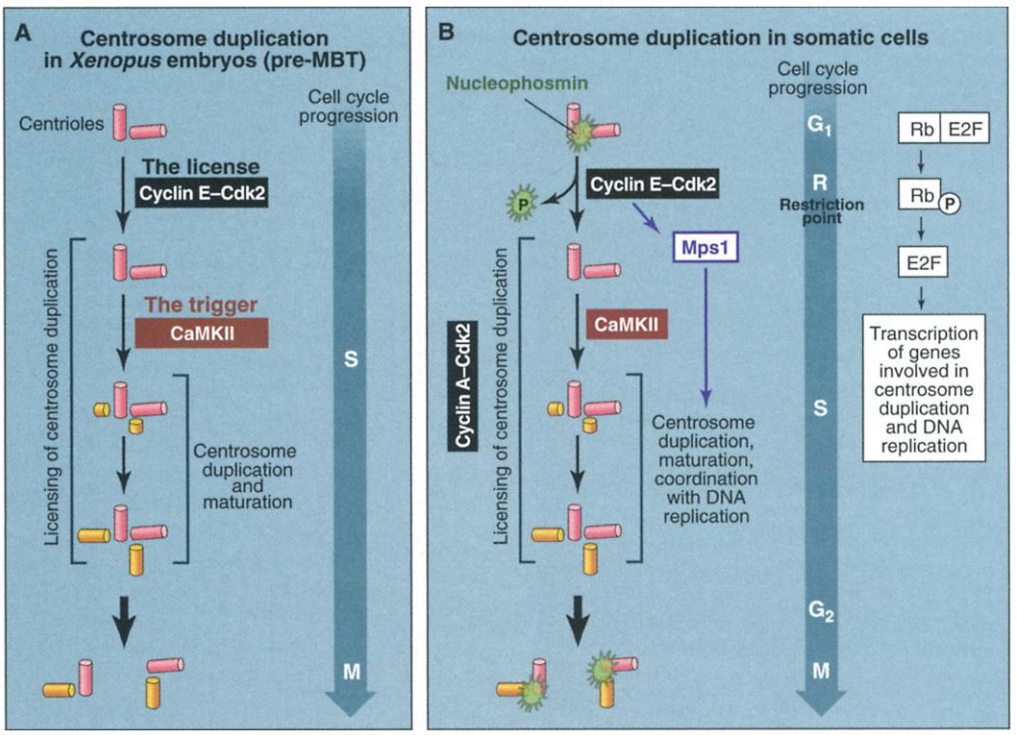
releases the transcription factor E2F, which moves to the nucleus and switches on genes required for S phase (see the figure). Normal coordination between centrosome duplication and DNA replication can be disrupted by treating cells with hydroxyurea, which induces cell cycle arrest in S phase. If cells are treated with hydroxyurea, DNA replication is blocked but the centrosome is duplicated normally or may even be amplified, leading to multiple copies (*6*). If an aberrant form of Cdk2 that overrides the activity of the wild-type kinase is overexpressed in hydroxyurea-treated cells, both DNA replication and centrosome duplication are abrogated (*7*). The targets of Cdk2 activity, which are involved in centrosome duplication but not DNA replication, include the nucleolar protein nucleophosmin (B23) (*8*) and the kinase Mps1p (*9*).



A license to duplicate. (**A**) In the midblastula (MBT) stage *Xenopus* embryo, duplication of the centrosome (composed of two centrioles; red) is licensed by cyclin E–Cdk2 and triggered by CaMKII. (**B**) In somatic cells, the situation is more complex because of the presence of cell cycle checkpoints. At the G_1-S phase transition (restriction point), centrosome duplication is licensed by cyclin E–Cdk2, which directly phosphorylates nucleophosmin (green). The cyclin E–Cdk2 complex may also be involved in phosphorylation of the Rb tumor suppressor protein, which then releases E2F. This transcription factor moves to the nucleus and switches on the expression of S-phase genes that direct centrosome duplication and DNA replication. Centrosome duplication is triggered by CaMKII, which is activated by a sudden increase in intracellular free calcium ions. The continuation of centrosome duplication during S phase depends on the cyclin A–Cdk2 complex. Coordination of the centrosome cycle with the nuclear cycle is controlled at multiple levels by the kinase Mps1p, which is under the control of Cdk2. Other kinases such as aurora-A might also be involved (*12*).

The unphosphorylated form of nucleophosmin associates with the single centrosome during G_1 (see the figure). Upon phosphorylation by the cyclin E–Cdk2 complex, nucleophosmin leaves the centrosome (*8*), triggering centrosome duplication. Most important, unphosphorylated nucleophosmin only reassociates with the centrosome toward the end of mitosis. A mutant form (Thr^{199}→Ala) of nucleophosmin that could not be phosphorylated by Cdk2 prevented centrosome duplication (*10*). It is also noteworthy that the cyclin A–Cdk2 complex phosphorylates Thr^{199} of nucleophosmin as efficiently as does cyclin E–Cdk2, and may sustain centrosome duplication during S phase. Thus, phosphorylated nucleophosmin is part of the molecular machinery that "authorizes" (licenses) centrosome duplication.

The kinase Mps1p is another key component of the Cdk2 pathway. Treating cells with hydroxyurea usually induces S phase arrest without centrosome amplification. But if Mps1p is overexpressed in hydroxyurea-treated cells, replication of the centrosome is amplified. If such cells are treated with roscovitin, which blocks Cdk2 activity, centrosome amplification is abrogated because Cdk2 is required for stabilization of Mps1p (*9*). The exact contribution of Mps1p to centrosome duplication remains to be determined.

Establishing that Cdk2 activity licenses centrosome duplication still does not reveal the actual trigger for this process. To identify the trigger, Matsumoto and Maller studied cell division in fertilized *Xenopus* frog eggs (*3*). Frog embryos do not acquire cell cycle checkpoints—cyclin E–Cdk2 activity is stable and nucleophosmin not yet made—until 12 cell divisions after fertilization, the midblastula stage. Despite the lack of checkpoints, centrosome duplication still has to be carefully regulated at each cell division.

In their *Xenopus* egg extracts, Matsumoto and Maller identified CaMKII, itself activated by a sudden increase (spike) in calcium ions, as the trigger for centrosome duplication. They demonstrate that CaMKII activity is required for each round of centrosome duplication, including that during the very first cell division of the fertilized eggs. Inhibition of Cdk2 did not affect the initial rounds of centrosome duplication. The identification of CaMKII as the trigger of centrosome duplication connects molecular events with the decades-old observation that each division of the frog embryo is accompanied by a large spike in free calcium ions.

By linking calcium ion release, calmodulin (a calcium binding protein), and CaMKII to centrosome duplication, Matsumoto and Maller have opened the door to many further investigations. Intriguingly, several calcium ion spikes, including one at the G_1-S boundary, have been observed during the cell cycle of cultured cells (*11*). The next steps will be to confirm that CaMKII is the trigger for centrosome duplication in somatic cells and to identify the targets of CaMKII within the centrosome.

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