Dark-maned males are presumably better able to withstand heat-related costs, and although we could find no heritability in darkness, mate choice for dark manes might confer indirect genetic benefits as well as direct fitness effects.

Heat appears to be the dominant ecological factor shaping the lion's mane. Mane length showed a significant relationship to annual T_a fluctuations from 1964 to 2000 (Table 1), and manes are darker in cooler habitats and seasons. Long-term climate forecasts predict an increase of 1.3° to 4.6°C in this region by the year 2080 (*38*); thus, manes are likely to become shorter and lighter in these populations. The general importance of ambient temperature to sexual selection is not yet known, and temperature effects may be most obvious in animals where large body size already imposes thermal stress. However, any indicator trait with high energetic costs should be sensitive to ambient temperature, suggesting broad implications for studies of sexual selection.

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25. Paternity was known for 13 sons of 6 males (*26*), but there was no relationship between the manes of fathers and sons. We also estimated heritability using average mane length and color of males in paternal coalitions [there is no extra-group paternity in lions (*26*)]. There was no correlation with paternal color for 68 adult sons. Although length was significant by univariate analysis ($T = 2.63$, $P = 0.0107$, $n = 68$), fathers and sons reside in similar habitats as adults, and the correlation disappeared in a multivariate analysis controlling for habitat (Table 1).
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32. These results might reflect variations in diet or vegetation. Food supply is most constant in the Crater, relatively stable in the Serengeti woodlands, and sporadic on the Serengeti plains (because of seasonal migratory patterns). However, males born in woodland prides grow manes as light as those of males that reside in plains prides as adults (Table 1). It has been suggested that shredding by thorn bushes reduces mane length, but males born in the woodlands maintained shorter manes regardless of adult habitat, whereas those that moved to the woodlands as adults had manes of average length.
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36. The full model includes age (Student's t test = –3.09, $P = 0.0114$), resident in Ngorongoro Crater (Student's t test = 5.94, $P = 0.0001$), and sperm count (Student's t test = –5.08, $P = 0.0005$). Data were reanalyzed from (*39*). None of these factors had a detectable effect on fertility.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5585/1339/DC1
Materials and Methods
Supporting Text
Figs. S1 and S2
Table S1
Movies S1 and S2

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Meiotic Arrest in the Mouse Follicle Maintained by a G_s Protein in the Oocyte

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The mammalian ovarian follicle consists of a multilayered complex of somatic cells that surround the oocyte. A signal from the follicle cells keeps the oocyte cell cycle arrested at prophase of meiosis I until luteinizing hormone from the pituitary acts on the follicle cells to release the arrest, causing meiosis to continue. Here we show that meiotic arrest can be released in mice by microinjecting the oocyte within the follicle with an antibody that inhibits the stimulatory heterotrimeric GTP–binding protein G_s. This indicates that G_s activity in the oocyte is required to maintain meiotic arrest within the ovarian follicle and suggests that the follicle may keep the cell cycle arrested by activating G_s.

Oocytes within mammalian ovarian follicles begin meiosis during embryogenesis but then arrest at prophase of meiosis I until luteinizing hormone acts on the follicle to cause meiosis to resume (*1*). Maintaining this arrest in fully grown oocytes depends on the presence of the surrounding follicle (Fig. 1, A and C); removing the oocyte from the follicle reinitiates meiosis. However, it is unknown how the follicle cells communicate with the oocyte to keep the cell cycle arrested. Signaling depends on maintaining a high level of adenosine 3′,5′-monophosphate (cAMP) in the oocyte, but where the cAMP comes from and how the follicle cells regulate its level is unclear (*1*). One hypothesis is that cAMP enters the oocyte through gap junctions with the follicle cells (*1*, *2*). Alternatively, cAMP could be generated in the oocyte, and the role of the follicle cells could be to maintain the activity of a stimulatory G protein (G_s) in the oocyte membrane, thus stimulating oocyte adenylyl cyclase (*1*, *3*). Although some evidence has been obtained for each model, neither possibility has been definitively tested.

Studies of how meiotic arrest is main-

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tained and released in mammalian oocytes have been limited by a technical problem: the oocyte is embedded in multiple layers of cells that must be left intact to preserve normal regulation. Here, we developed a method for injecting meiotically competent mouse oocytes within antral follicles (260 to 470 μm diameter). The follicle was slightly compressed between two coverslips separated by a 300-μm spacer; this assembly was mounted over a reservoir of medium on a support slide with a coverslip base (Fig. 1B) (*4*, *5*). The chamber was open at the front to allow introduction of a micropipette. The nucleus (germinal vesicle; GV) could be seen in favorable cases, and the nucleolus was always visible (Fig. 1C). The success of the injection was confirmed by including a fluorescent dextran in the injection solution (Fig. 1D).

To examine whether a G_s protein within the oocyte is required to maintain meiotic arrest within the follicle, we microinjected oocytes with an affinity-purified antibody that inhibits G_s function (*4*, *6*–*8*). This antibody was made against the COOH-terminal 10 amino acids of the α subunit of G_s and specifically recognized both the long and the short forms of G_s (*9*) in isolated oocytes (Fig. 2A). The amount of G_s protein in oocytes was comparable to that in brain (Fig. 2A), which indicates that G_s is present at a physiologically significant level.

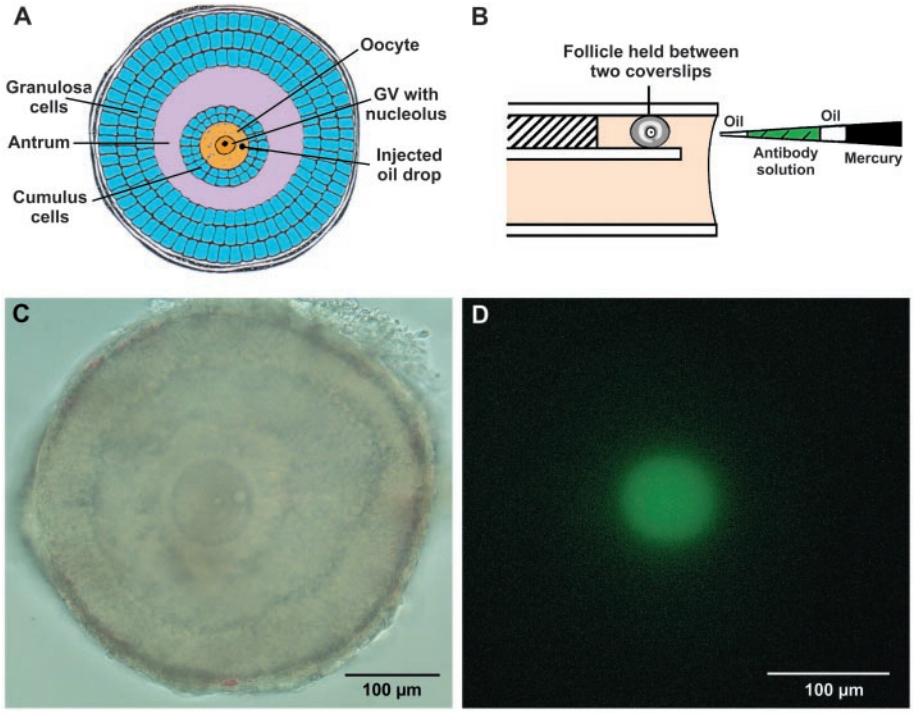

Fig. 1. A method for injecting follicle-enclosed oocytes. (**A**) Structure of the follicle, also showing an oil drop within the oocyte introduced by microinjection. Drawing modified from (*15*). (**B**) Injection chamber and micropipette (*4*). (**C**) A follicle containing an injected oocyte. The light spot near the oocyte center is the nucleolus; the light spot near the oocyte periphery is an oil drop introduced by the injection; see (A). (**D**) Fluorescence image of another follicle containing an injected oocyte; fluorescent dextran in the oocyte cytoplasm indicates a successful injection.

The G_s antibody caused resumption of meiosis in follicle-enclosed oocytes, as indicated by light microscopic observation that GV breakdown (GVBD) had occurred in oocytes that were removed from their follicles 3 hours after injection (Fig. 2B). The intracellular concentration of the G_s antibody required to cause GVBD in 50% of the oocytes was between 0.3 and 1.3 μM. Antibody-injected oocytes that underwent GVBD subsequently formed a first polar body (Fig. 2C), which indicates that meiosis progressed normally (14 of 16 oocytes observed).

Control oocytes that were injected with an antibody (6.7 μM) against another G protein, G_{i3} (*6*) (Fig. 2A), and other control oocytes that were not injected were GV intact when removed from their follicles 3 hours after injection (Fig. 2, B and D). The G_i antibody-injected oocytes, as well as control uninjected oocytes, underwent GVBD by 3 hours after removal from the follicle, which indicates that the G_i antibody itself had no detectable effect on oocyte maturation.

These experiments indicated that inhibition of G_s in the oocyte is sufficient to release meiotic arrest and, conversely, that G_s activity in the oocyte is required to maintain meiotic arrest within the follicle. Because the ~150-kD antibody is too large to pass into follicle cells through gap junctions, which generally have a permeability limit of ~1 kD (*10*), the antibody's inhibitory action must be on G_s in the oocyte and not in the follicle cells. It is possible, however, that cAMP entering the oocyte through gap junctions also contributes to the maintenance of meiotic arrest.

Oocytes that are removed from their follicles and maintained in a cAMP phosphodiesterase inhibitor such as hypoxanthine stay arrested at meiotic prophase (*1*, *3*, *4*), which suggests that isolated oocytes might have enough G_s activity to keep cAMP elevated if its hydrolysis is prevented. To examine this, we injected isolated oocytes with the G_s antibody and cultured them in the presence of hypoxanthine. GVBD occurred in these oocytes but not in controls injected with the G_i antibody (Fig. 2E), which indicates that, even in the absence of an intact follicle, at least some G_s activity remains in the oocyte (*4*). Additional controls showed that the G_s antibody did not cause GVBD by an action downstream of cAMP and that G_s antibody-matured oocytes responded normally to fertilization (*4*) (fig. S1).

G_s alone has little constitutive activity (*11*), which suggests that G_s activity in the oocyte might be maintained by a G_s-linked receptor in the oocyte membrane. Because G-protein–linked receptors can have high constitutive activity (*12*, *13*), this could potentially account for the G_s activity in isolated oocytes. In the intact follicle, a ligand might activate such a receptor further, possibly explaining the maintenance of meiotic arrest by the follicular environment (*4*). It is also possible that G_s activity in the oocyte is the same regardless of whether the follicle is present and that the meiosis-inhibiting signal from the follicle acts at another level of the cAMP pathway—for example, to suppress cAMP phosphodiesterase activity in the oocyte (*1*, *14*).

Comparing these findings with previous studies of frog (*Xenopus laevis*) oocytes, in which the same G_s antibody was found to cause GVBD (*8*), indicates that G_s activity may be a conserved mechanism for maintaining meiotic arrest in vertebrate oocytes (*4*) (fig. S2). A difference between mouse and *Xenopus*, however, is that *Xenopus* oocytes do not undergo spontaneous GVBD when removed from their follicles. This could be due to species differences in the level of constitutive activity of G_s-linked receptors in the oocyte membrane or of phosphodiesterase activity in the oocyte. By identifying oocyte G_s as a required factor in maintaining meiotic arrest, our results open the way for future investigations

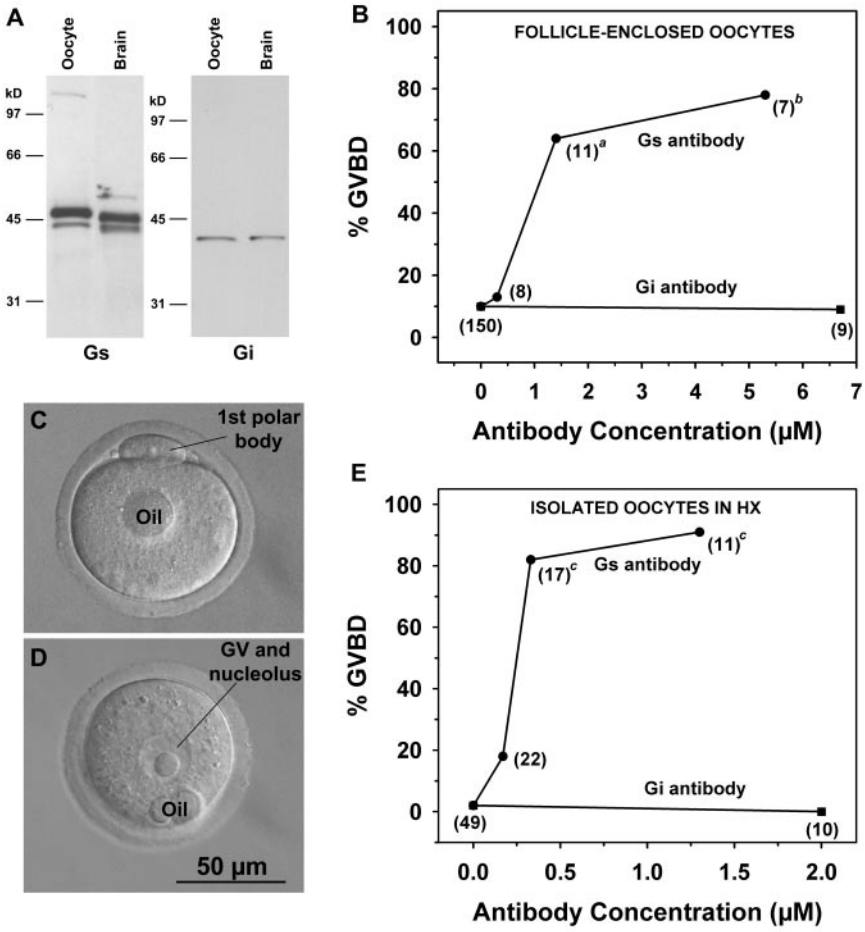

Fig. 2. Injection of an antibody against G_s causes oocyte maturation. (**A**) Immunoblots showing G_s and G_i protein in mouse oocytes and brain probed with the same antibodies used for microinjection. Total protein per lane = 4, 2.5, 5, and 5 μg for lanes 1 to 4, respectively. (**B**) Follicle-enclosed oocytes were injected with an antibody against G_s or with a control antibody against G_i; the graph shows antibody concentrations in the cytoplasm (the 0 μM point describes uninjected oocytes from follicles processed in parallel). Three hours later, the oocytes were removed from their follicles and scored for the presence or absence of a nucleus (% GVBD). (**C**) Formation of a polar body by an oocyte injected with the G_s antibody (1.3 μM). The oocyte was removed from the follicle 3 hours after injection, at which time it had undergone GVBD; it was photographed 20 hours later. (**D**) Control oocyte injected with the G_i antibody (6.7 μM) and removed from the follicle 3 hours later, at which time the GV was intact. (**E**) Isolated oocytes were injected with antibodies against G_s or G_i, incubated in the presence of 4 mM hypoxanthine (Hx), and scored for GVBD 3 hours later. For (B) and (E), numbers in parentheses indicate the number of oocytes injected with each antibody concentration, and superscript letters indicate the statistical significance of the results compared with G_i antibody-injected controls (*a*, $P < 0.03$; *b*, $P < 0.01$; *c*, $P < 0.001$; Fisher's exact test) (*4*).

of signaling molecules from the follicle that maintain meiotic arrest in the oocyte as well as the mechanism by which luteinizing hormone causes the resumption of meiosis.

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Materials and Methods
SOM Text
Figs. S1 and S2
References

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Distinct Modes of Signal Recognition Particle Interaction with the Ribosome

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Signal recognition particle (SRP), together with its receptor (SR), mediates the targeting of ribosome-nascent chain complexes to the endoplasmic reticulum. Using protein cross-linking, we detected distinct modes in the binding of SRP to the ribosome. During signal peptide recognition, SRP54 is positioned at the exit site close to ribosomal proteins L23a and L35. When SRP54 contacts SR, SRP54 is rearranged such that it is no longer close to L23a. This repositioning may allow the translocon to dock with the ribosome, leading to insertion of the signal peptide into the translocation channel.

Secretory proteins are synthesized with an NH_2-terminal hydrophobic signal peptide, which is recognized by the SRP as it emerges from the ribosome (*1*, *2*). SRP targets the ribosome, together with the associated nascent chain, to the endoplasmic reticulum (ER) via