serotonin system acts as a unitary broadcast system conveying a unified signal, 'patience', to the rest of the brain. On the other hand, single unit recording studies have demonstrated that dorsal raphe nucleus neurons in general and pharmacologically identified dorsal raphe nucleus serotonin neurons in particular display a rich diversity of responses to behaviorally salient stimuli, movement as well as reward [7,9–13]. Reward is particularly interesting, because recording studies have unanimously found that the firing of serotonin neurons strongly correlates with reward-related variables such as reward delay, reward amount and reward timing. Moreover, serotonin depletion compromises reward processing in humans and animals [14].

In fact, a recent seminal study by Liu *et al.* [13] reached a different conclusion: dorsal raphe nucleus serotonin neurons encode reward signals and drive learning. Employing a similar optogenetic strategy to achieve spatiotemporal and genetic specificity, Liu *et al.* [13] obtained strong causal evidence that the activation of dorsal raphe nucleus serotonin neurons reinforced mice to stay longer in the stimulation-coupled locations, shifted sucrose preference, drove optical self-stimulation, and directed sensory discrimination learning. These results suggest that dorsal raphe nucleus serotonin neurons signal reward and reinforce behaviors.

These seemingly disparate findings may be attributed simply to differences in optogenetic reagents, the degree and pattern of activation or different promoters used that may target partially non-overlapping dorsal raphe nucleus serotonin neuron populations. Alternatively, the apparent differences between the two sets of results [1,13] may reflect distinct signaling regimes of dorsal raphe nucleus serotonin neurons. The current study produced a sustained increase in firing mimicking a 'tonic' mode of activation. In contrast, Liu *et al.* [13] used a transient and more synchronous mode of stimulation representing a 'phasic' mode. These authors demonstrated that some of the reinforcing effects of phasic stimulation are achieved through the activation of midbrain dopamine neurons. In contrast, the tonic mode in the current study may modulate activity in different target regions such as the medial prefrontal cortex. This hypothesis would be similar to the differential impact of phasic and tonic dopamine signals [15–19].

These exciting developments re-emphasize the multifaceted nature of the serotonin system. Reflecting on the success of computational models in elucidating the function of dopamine in learning and actions [20], we hope to see future research lead to a theoretical framework that embraces these seemingly orthogonal concepts of patience and reward [16,18].

References

1. Miyazaki, K.W., Miyazaki, K., Tanaka, K.F., Yamanaka, A., Takahashi, A., Tabuchi, S., and Doya, K. (2014). Optogenetic activation of dorsal raphe serotonin neurons enhances patience for future rewards. Curr. Biol. *24*, 2033–2040.
2. Mobini, S., Chiang, T.J., Al-Ruwaitea, A.S., Ho, M.Y., Bradshaw, C.M., and Szabadi, E. (2000). Effect of central 5-hydroxytryptamine depletion on inter-temporal choice: a quantitative analysis. Psychopharmacology *149*, 313–318.
3. Eagle, D.M., Lehmann, O., Theobald, D.E.H., Pena, Y., Zakaria, R., Ghosh, R., Dalley, J.W., and Robbins, T.W. (2009). Serotonin depletion impairs waiting but not stop-signal reaction time in rats: implications for theories of the role of 5-HT in behavioral inhibition. Neuropsychopharmacology *34*, 1311–1321.
4. Doya, K. (2002). Metalearning and neuromodulation. Neural Netw. *15*, 495–506.
5. Schweighofer, N., Bertin, M., Shishida, K., Okamoto, Y., Tanaka, S.C., Yamawaki, S., and Doya, K. (2008). Low-serotonin levels increase delayed reward discounting in humans. J. Neurosci. *28*, 4528–4532.
6. Miyazaki, K.W., Miyazaki, K., and Doya, K. (2012). Activation of dorsal raphe serotonin neurons is necessary for waiting for delayed rewards. J. Neurosci. *32*, 10451–10457.
7. Miyazaki, K., Miyazaki, K.W., and Doya, K. (2011). Activation of dorsal raphe serotonin neurons underlies waiting for delayed rewards. J. Neurosci. *31*, 469–479.
8. Berndt, A., Yizhar, O., Gunaydin, L.A., Hegemann, P., and Deisseroth, K. (2009). Bi-stable neural state switches. Nat. Neurosci. *12*, 229–234.
9. Ranade, S.P., and Mainen, Z.F. (2009). Transient firing of dorsal raphe neurons encodes diverse and specific sensory, motor, and reward events. J. Neurophysiol. *102*, 3026–3037.
10. Nakamura, K., Matsumoto, M., and Hikosaka, O. (2008). Reward-dependent modulation of neuronal activity in the primate dorsal raphe nucleus. J. Neurosci. *28*, 5331–5343.
11. Bromberg-Martin, E.S., Hikosaka, O., and Nakamura, K. (2010). Coding of task reward value in the dorsal raphe nucleus. J. Neurosci. *30*, 6262–6272.
12. Li, Y., Dalphin, N., and Hyland, B.I. (2013). Association with reward negatively modulates short latency phasic conditioned responses of dorsal raphe nucleus neurons in freely moving rats. J. Neurosci. *33*, 5065–5078.
13. Liu, Z., Zhou, J., Li, Y., Hu, F., Lu, Y., Ma, M., Feng, Q., Zhang, J.-E., Wang, D., Zeng, J., *et al.* (2014). Dorsal raphe neurons signal reward through 5-HT and glutamate. Neuron *81*, 1360–1374.
14. Seymour, B., Daw, N.D., Roiser, J.P., Dayan, P., and Dolan, R. (2012). Serotonin selectively modulates reward value in human decision-making. J. Neurosci. *32*, 5833–5842.
15. Fiorillo, C.D., Tobler, P.N., and Schultz, W. (2003). Discrete coding of reward probability and uncertainty by dopamine neurons. Science *299*, 1898–1902.
16. Cools, R., Nakamura, K., and Daw, N.D. (2011). Serotonin and dopamine: unifying affective, activational, and decision functions. Neuropsychopharmacology *36*, 98–113.
17. Niv, Y., Daw, N.D., Joel, D., and Dayan, P. (2007). Tonic dopamine: opportunity costs and the control of response vigor. Psychopharmacology *191*, 507–520.
18. Daw, N.D., Kakade, S., and Dayan, P. (2002). Opponent interactions between serotonin and dopamine. Neural Netw. *15*, 603–616.
19. Tsai, H.C., Zhang, F., Adamantidis, A., Stuber, G.D., Bonci, A., de Lecea, L., and Deisseroth, K. (2009). Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science *324*, 1080–1084.
20. Schultz, W., Dayan, P., and Montague, P.R. (1997). A neural substrate of prediction and reward. Science *275*, 1593–1599.

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Sexual Selection: Placentation, Superfetation, and Coercive Copulation

The evolution of placentas in poeciliid fishes is associated with conception of overlapping litters and male mating strategies becoming more coercive. Sperm competition in ovaries of multiply-inseminated females may favor fertilization of immature eggs during ongoing pregnancies.

David Haig

Intersexual selection is commonly described as the process by which female choice of mating partners shapes male attributes to conform to female preferences. However, it also encompasses male adaptations to circumvent female choice by deceit or coercion. The diverse life histories of

Figure 1. Maternal provisioning, courtship and coercion in poeciliid fishes.

(A) A colorful *Poecilia latipinna* male courts a lecithotrophic female (photo courtesy of Don DeMaria). (B) A drab *Heterandria formosa* male with a long gonopodium (photo courtesy of Pierson Hill). (C) A male with gonopodium swung forward approaches a matrotrophic *Heterandria* female (photo courtesy of Chiara Sciarone).

fish provide many opportunities for exploring this evolutionary dynamic. External fertilization allows a female control over who sires her fry because she determines when (and near whom) her eggs are released. But in many species, the non-chosen males adopt opportunistic strategies of darting in to release sperm at the moment a female spawns with a chosen male [1]. Internal fertilization has evolved multiple times in fishes, perhaps as an adaptation to preempt sperm of other males by fertilizing eggs before their release. Males gain the additional benefit that they need not wait until females oviposit but can deposit their sperm and leave in search of other females. And males can inseminate without being chosen. Copulation probably began as an assertion of male priorities over female autonomy [2]. A recent study by Pollux *et al.* [3] finds surprising correlates of male mating behavior in guppies and their relatives (poeciliid fishes).

Poeciliid males use an elaborately modified anal fin, a gonopodium, to inject sperm into female gonoducts, and are noted for bright colors, elaborate courtship and frequent coercive mating [3]. Males of some species (e.g., *Poecilia latipinna*; Figure 1A) court females or sneak copulations depending on male genotype and opportunity [4] whereas males of other species (e.g., *Heterandria formosa;* Figure 1B,C) rely exclusively on unsolicited copulation without prior courtship [5]. Fertilization and embryonic development occur within ovarian follicles, with well-developed offspring being released from the follicles shortly before birth. The timing of maternal investment varies between species: in some, eggs are fully provisioned with yolk before fertilization (lecithotrophy), whereas in others mothers transfer substantial nutrients to embryos after fertilization (matrotrophy). Nutrients are transferred across follicular epithelia of both lecithotrophic and matrotrophic mothers but transfer is considered to be ‘placental’ once follicles contain embryos.

Pollux *et al.* [3] studied associations between placentation and male secondary sexual characters in 94 species of poeciliid fish. Matrotrophy was associated with loss of bright colors and courtship displays in males, suggesting reduced importance of precopulatory female choice. Matrotrophy was also

associated with smaller males with longer gonopodia, traits that enhance male manouverability and success in rapid copulatory forays. Finally, matrotrophy was associated with superfetation — the presence of multiple broods at different stages of development within individual ovaries. Thus, post-zygotic maternal provisioning is associated with shifts in male behavior from courtship to coercion [3].

The authors propose that matrotrophy and superfetation create opportunities for post-copulatory choice by females and thus lead to a loss of courtship by males [3]. An alternative interpretation reverses the causal arrow: matrotrophy and superfetation are consequences of changes in male behavior. The difference between these hypotheses is expressed in the question, did males abandon courtship and bright colors because females ceased to prefer these traits, or did males cease to court because of increased returns from coercion? In other words, did placentation enable a shift in female criteria of choice from pre-copulatory to post-copulatory characters, or was placentation an indirect consequence of changes in male behavior that reduced female opportunities of pre-copulatory choice?

Female poeciliids need not remate to replenish sperm after giving birth. In a recent field study [6], many guppies were fathered posthumously by dead males whose sperm had survived for months in the ovaries of longer-lived females. Thus, sperm from a single mating can survive through multiple pregnancies to sire offspring in multiple litters, and the ovaries of pregnant females contain resident sperm waiting for eggs to fertilize. In some lecithotrophic poeciliids, the next clutch does not start to fill with yolk until after the birth of the previous litter, whereas vitellogenesis in other species commences during pregnancy with fertilization of a new clutch immediately after birth of the previous litter [7].

Matrotrophy and superfetation may have originated from sperm 'jumping the gun' and fertilizing eggs before the eggs were fully provisioned with yolk. Such a strategy could reflect competition among sperm already present in ovaries or preemptive action of sperm from earlier inseminations to forestall fertilizations by sperm of future inseminations from other males. If a sperm fertilizes an egg before it is fully-yolked and the mother continues to provision its follicle, then the latter stages of provisioning are post-zygotic (matrotrophic) rather than pre-zygotic (lecithotrophic) without a change of maternal physiology. If eggs are fertilized while a mother is pregnant, she carries overlapping litters without any change in the way she provisions follicles (Figure 2). Although superfetation and matrotrophy may have originated from 'premature' fertilization of immature eggs, the expression of these characters in extant species will have been modified by subsequent selection on maternal supply and offspring demand.

These hypotheses do not explain directly why matrotrophy and superfetation should be associated with coercive mating. A possible explanation is that more male investment in coercion results in more intense sperm competition because females are inseminated by more males. The evolution of superfetation is probably also facilitated by selection on females for rapid production of offspring, favoring maturation of the next clutch of oocytes during an ongoing pregnancy. One curious consequence of coercive mating is that it may have facilitated the evolution of female-only lineages (e.g., *Poecilia formosa*, *Poeciliopsis monacha-lucida*) that use sperm from males of related species to sire offspring but pass on their maternal genes only [8]. Copulation with these females is a genetic dead end for males but the need for coercive males to make quick decisions without close inspection may aid their deception by female 'sperm-parasites'.

More than a century ago, Seal described the mating behavior of the fish *Gambusia holbrooki* and *Heterandria formosa* [5]. He wrote that "The males are continually engaged in a pursuit of the females while the females are apparently adverse to sexual dalliance and at all times unwilling participators and quick to resent the advances of the males. I have never witnessed anything to indicate a reciprocity of desire in coitus it being always a chance touch and go on the part of the males." But he also described males fleeing in terror from the much larger females who would sometimes kill their sexual harassers. He observed that "in the attacks of the females of either species they seem to endeavor to bite the long slender organ of the male, which is no doubt the most vulnerable point." Females are neither behaviorally nor evolutionarily passive.

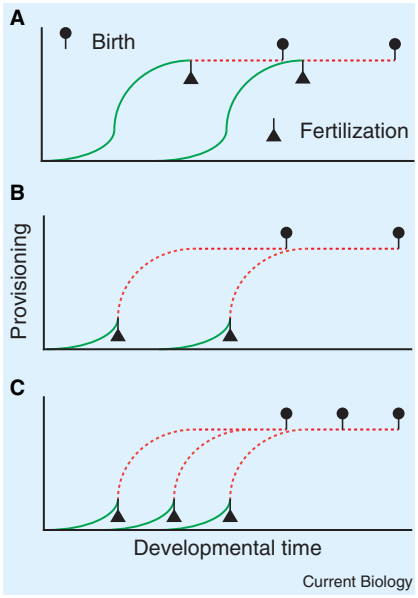

Figure 2. Matrotrophy and superfetation can originate from shifts in the timing of fertilization.

(A) An ancestral lecithotrophic species provisions oocytes before fertilization (green line). Fetal development (dotted red line) continues without further increase in weight. (B) Eggs are fertilized before they are fully provisioned. As a result, provisioning continues after fertilization (matrotrophy) and the next litter is conceived before birth of the previous litter (superfetation). (C) Subsequent evolution results in multiple small litters with short interbirth intervals.

Consensual mating becomes more attractive for males when female adaptations reduce relative returns from coercion. By this process, mating systems can evolve to be less coercive. Phylogenetic analyses suggest that coercion is ancestral for male poeciliids and that courtship has evolved and been lost multiple times [9]. Females are proposed to obtain genetic benefits from mating with multiple males via post-copulatory choice of which sperm fertilize their eggs or which offspring they provision [10–13]. But males may evolve adaptations to subvert these mechanisms, and polyandry need not be adaptive for females if females cannot choose their sexual 'partners'.

References

1. Taborsky, M. (1998). Sperm competition in fish: 'bourgeois' males and parasitic spawning. Trends Ecol. Evol. *13*, 222–227.

2. Dworkin, A. (1987). Intercourse (New York: Free Press).
3. Pollux, B.J.A., Meredith, R.W., Springer, M.S., and Reznick, D.N. (2014). The evolution of the placenta drives a shift in sexual selection in livebearing fish. Nature, in press.
4. Fraser, B.A., Janowitz, I., Thairu, M., Travis, J., and Hughes, K.A. (2014). Phenotypic and genomic plasticity of alternative male reproductive tactics in sailfin mollies. Proc. R. Soc. B *281*, 20132310.
5. Seal, W.P. (1911). Breeding habits of the viviparous fishes *Gambusia holbrookii* and *Heterandria formosa*. Proc. Biol. Soc. Washington *24*, 91–96.
6. López-Sepulcre, A., Gordon, S.P., Paterson, I.G., Bentzen, P., and Reznick, D.N. (2014). Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. Proc. R. Soc. B *280*, 20131116.
7. Turner, C.L. (1937). Reproductive cycles and superfetation in poeciliid fishes. Biol. Bull. *72*, 145–164.
8. Vrijenhoek, R.C. (1994). Unisexual fish: model systems for studying ecology and evolution. Annu. Rev. Ecol. Syst. *25*, 71–96.
9. Bisazza, A., Grapputo, A., and Nigro, L. (1997). Evolution of reproductive strategies and male sexual ornaments in poeciliid fishes as inferred by mitochondrial 16 rRNA gene phylogeny. Ethol. Ecol. Evol. *9*, 55–67.
10. Jennions, M.D., and Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. Biol. Rev. *75*, 21–64.
11. Eberhard, W.G. (2001). Postcopulatory sexual selection: Darwin's omission and its consequences. Proc. Natl. Acad. Sci. USA *106*, 10025–10032.
12. Zeh, J.A., and Zeh, D.W. (2001). Reproductive mode and the genetic benefits of polyandry. Anim. Behav. *61*, 1051–1063.
13. Simmons, L.W. (2005). The evolution of polyandry: sperm competition, sperm selection, and offspring viability. Annu. Rev. Ecol. Evol. Syst. *36*, 125–146.

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Centromere Licensing: Mis18 Is Required to Polo-ver

The Mis18 complex is a critical player in determining when and where centromeres are built. A new study identifies Polo-like kinase (Plk1) as a positive regulator required for the localization of Mis18 to centromeres. This is a critical step that is essential for proper centromere function and maintaining the integrity of the genome.

Meghan C. Barnhart-Dailey[1] and Daniel R. Foltz[1,2,*]

In order to accurately transmit genetic information to daughter cells during mitotic division, vertebrate cells must maintain a single centromere on each chromosome. The centromere is the chromatin site on which the kinetochore will assemble during mitosis and will attach the chromosome to the mitotic spindle. The key determinant of centromere position in most eukaryotes is the presence of the centromere-specific histone H3 variant CENP-A. The site of centromere formation and CENP-A deposition is determined epigenetically in higher eukaryotes, depending on the proteins present at the centromere but not the underlying DNA sequence [1–3]. A new study by McKinley *et al.* [4] published recently in *Cell* provides important insight into how centromere assembly is controlled by demonstrating that the mitotic kinase Plk1 is a positive regulator of new CENP-A deposition.

Centromeric CENP-A nucleosomes are highly stable and are quantitatively retained during replication of centromeric DNA in S-phase [5,6]. The redistribution of CENP-A nucleosomes between the two new DNA strands is necessary to maintain the epigenetic mark of the centromere and leads to the dilution of CENP-A nucleosomes. Therefore, new CENP-A nucleosomes must be assembled during each cell cycle to maintain CENP-A at centromeres, and to ensure that the epigenetic mark is not lost over multiple generations. Canonical histone H3.1 nucleosomes contained within general chromatin are restored to their full complement on each daughter strand during DNA replication [3]. However, CENP-A nucleosomes are not deposited concurrently with DNA replication. Instead, new CENP-A nucleosomes are deposited in early G1 in vertebrate cells [5].

Centromere assembly is thought to be controlled by a process of licensing that restricts the assembly of new CENP-A to the site of the existing centromere. This process relies on temporal control of new CENP-A deposition restricting it to G1. The unique timing of CENP-A deposition suggests a novel temporal control mechanism that is linked to the progression of cells through mitosis. Two kinases appear to provide positive and negative regulation of new CENP-A deposition to ensure it occurs exclusively in G1 phase. The new findings by McKinley *et al.* [4] reveal that the mitotic Polo-like kinase 1 (Plk1) is required for new CENP-A deposition in early G1. This complements earlier studies where CDK1 activity was shown to negatively regulate CENP-A deposition [7]. CDK1 activity prevents deposition from occurring prior to completion of mitosis, after which time Plk1 takes over to activate new CENP-A deposition.

Two of the factors known to be required for orchestrating CENP-A deposition in human cells are the Mis18 complex and the CENP-A-specific chaperone, Holliday junction recognition protein (HJURP) [8–11]. The Mis18 complex is composed of Mis18α, Mis18β, and M18BP1 (also known as Mis18BP1 or hsKNL2) in human cells, and is recruited to centromeres beginning in late telophase and persists through early G1 [8]. Mis18 localizes to centromeres just prior to the pre-nucleosomal HJURP/CENP-A/H4 complex and is absolutely required for HJURP to reach centromeres [12,13].

Work by the Cheeseman and Jansen labs together demonstrated that a key event in controlling the timing of CENP-A deposition is the regulation of Mis18 complex localization by phosphorylation. Silva *et al.* demonstrated that phosphorylation of M18BP1 by CDK1 and CDK2 negatively regulates M18BP1's ability to localize to centromeres. Inhibiting CDK activity caused premature Mis18 complex loading onto centromeres in G2 and resulted in early CENP-A deposition [7]. The negative regulation of Mis18 complex localization by CDK phosphorylation agrees with the observed Mis18 complex localization at centromeres only after anaphase onset when CDKs are rapidly degraded and no longer present to phosphorylate Mis18.