A: Which approach to overcoming the problems of pleiotropy in mutant screens would be better able to recover mutations in genes that are NOT cell autonomous in their action: sensitization or mitotic recombination? Explain briefly.

B: Shown below are eight different genotypes depicting a homologous pair of autosomes, with the following symbol conventions: **FRT sites (#); centromeres (o); and a loss-of-function Minute mutant allele (M).** Minutes are cell-autonomous haploinsufficient genes that are essential for cell survival and growth. Since cells that are heterozygous for Minute loss-of-function alleles (M/) grow more slowly than their +/+ neighbors, mitotic recombination in a M/+ heterozygote can produce a M+/M+ clone that will have a growth advantage over its M/+ neighbors. M mutations are often included in clonal genetic screens, since if the newly induced recessive alleles to be screened are induced on the M+ chromosome, the M+/M+ clones induced will not only be homozygous for any new mutant alleles, they will also have a potential growth advantage over their M/+ neighbors. This growth advantage may make the clones larger and hence easier to score than would be the case in a background with no M mutation present. Moreover, the growth handicap of the cells outside the clone can reduce the competitive pressures among cells and thereby allow homozygous mutant cells to survive that would otherwise be unable to compete with +/+ neighbors due to adverse effects of the new mutation on growth. Below if no M is shown, assume that the allele present is wildtype.

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A: xxx(#)xxx(M)xxxxx(#)xxx(o)xxxxxx
xxxxxxxxxxxxxxxxxxx(o)xxxxxx

B: xxx(#)xxxxxxxxxxxx(#)(o)xxxxxx
xxxxxxxxxxxxxxxxxxx(o)xxxxxx

C: xxx(M)xxxxxxxxxxxx(#)(o)xxxxxx
xxxxxxxxxxxxxxxxxxxx(o)xxxxxx

D: xxx(#)xxxxxxxxxxxx(M)(o)xxxxxx
xxxxxxxxxxxxxxxxxxxx(o)xxxxxx

E: xxx(M)xxxxxxxxxxxx(#)(o)xxxxxx
xxxxxxxxxxxxxxxxxxxx(o)xxxxxx

F: xxx(#)xxxxxxxxxxxx(M)(o)xxxxxx
xxxxxxxxxxxxxxxxxxxx(o)xxxxxx

G: xxx(M)xxxxxxxxxxxx(#)(o)xxxxxx
xxxxxxxxxxxxxxxxxxxx(o)xxxxxx

H: xxx(M)xxxxxxxxxxxxx(o)x(#)xx
xxxxxxxxxxxxxxxxxxxxx(o)x(#)xx
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B-A: Which, if any, of these arrangements of FRT sites and M alleles could be used for an efficient mitotic recombination screen to identify carriers of
recessive mutations on the left arm of the chromosome shown that affect
the orientation of cell divisions? (no explanation necessary)
B-B: Can you deduce which parent was mutagenized to produce the genotype
you chose in part A -- the one carrying the *Minute* mutation or the one
that did not? Explain briefly.
B-C: What additional genetic element or combination of elements is necessary
to make the genotype you choose work for a mutant screen?

C: *Drosophila* geneticists try to minimize the work needed to maintain the many lines
of flies they investigate. They like to establish genetic stocks in which the flies in a
culture bottle randomly mating with each other produce progeny that are just like their
parents, generation after generation, with no further intervention by the geneticist.
Because females homozygous for a strict maternal-effect lethal (*mel*) mutation are
sterile (their offspring die during embryogenesis, regardless of their genotype with
respect to *mel*), such mutations can be propagated only when females are
heterozygous. A particular *mel* mutation on the second chromosome (an autosome) is
kept as a heterozygote with a balancer chromosome carrying the *Cy* mutation, which is
recessive (embryonic) lethal but also produces a dominant Curly-wing phenotype.
C-A: When this balanced stock reaches equilibrium, do you expect there to be
more *CyO/mel* males than *mel/mel* males? Explain briefly.
C-B: When this balanced stock reaches equilibrium, do you expect there to be
more *mel/mel* males than *mel/mel* females? Explain briefly
C-C: What kinds of flies would you select from the culture bottle to study the
phenotypic effect of the maternal-effect mutation in detail and how would
you recognize them?

D: The snarg is a hypothetical model diploid genetic organism that normally has seven
legs in both sexes. Snargs have heteromorphic sex chromosomes, with the male being
the homogametic sex. Male snargs homozygous for the amorphic (null) allele *leggy*\(^{12}\)
(*lgy*\(^{12}\)) have twelve legs, while males that are heterozygous (*lgy*\(^{12}/+\)) have nine legs.
Snargs only carry a single copy of the *lgy* gene. Somewhat surprisingly,
hemizygous *lgy*\(^{12}\) mutant female snargs have fifteen legs. The *lgy*\(^{12}\) mutant phenotypes
are not affected by temperature.
A dominant, partially temperature-conditional allele, *lgy*\(^D\), was discovered that,
whether heterozygous, homozygous, or hemizygous, produces animals with three legs
when those individuals are grown at 30°C, but five legs if they are grown instead at
20°C, regardless of sex. *lgy*\(^D/lgy*\(^{12}\) males have the same phenotype as *lgy*\(^D/+\) males.
D-A: Does the snarg appear to have a ZZ/ZW or instead an XX/XY system of
sex determination? Explain briefly.
D-B: Is there any indication that Muller's ratchet has been operating in the snarg
over evolutionary time? Explain briefly.
D-C: The fact that *lgy*\(^{12}/+\) males are not wildtype shows that the *lgy* gene
belongs to a rather exclusive (relatively few members) category of genes.
What is this category?
D-D: What, if any, evidence argues for or against snargs having a system of
sex-chromosome dosage compensation? Explain, briefly.
D-E: What category of gain-of-function allele does \( lgy^D \) appear to be? Explain briefly.

D-F: Although \( lgy^D \) does not show the kind of ideal temperature-sensitive behavior we might like to see, which growth temperature can we say is the more "restrictive" (or "non-permissive")?

D-G: How many legs would a \( lgy^D/+ \) male snarg have when grown at 20 C if this dominant allele were displaying the most clear-cut kind of temperature-sensitivity that we like to work with?

D-H: If we did a series of single temperature shifts (that is, start growth at either the restrictive or permissive temperature, then shift to the opposite temperature once during the development of the snarg), what specific change in leg phenotype would signal the beginning of the temperature-sensitive period for \( lgy^D/+ \) males?

D-I: What phenotype would you expect for the cis arrangement of these two mutations: \( lgy^D \& 12/lgy^+ \). Be sure to mention any temperature effects you might expect for this genotype.

D-J: What phenotype would you expect for \( lgy^D \& 12/lgy^-D \& 12 \) males (that is, males homozygous for the doubly-mutant allele)? Again, be sure to mention any temperature effects that you might expect for this genotype.

D-K: Is the difference in leg number between \( lgy^{12} \) homozygous males vs. \( lgy^{12} \) hemizygous females somewhat surprising? Explain briefly.

E: You were told that fruit flies produced from fathers who were exposed to ethylmethanesulfonate (the most common and effective mutagen for this organism) are often genetic mosaics due to the fact that only one of the two strands of DNA in the sperm is likely to be chemically modified at any particular base pair along the chromosome. You were also told that we can use various tricks for making genetic mosaics to greatly improve the efficiency of genetic screens -- most importantly, in those cases where mosaicism is induced in the F1 generation following exposure of the fathers' sperm to mutagen. Why do we have to use various tricks (like the FLP/FRT system) to make mosaics for these F1 genetic screens when the EMS mutagen itself already makes mosaics?

F. For blowflies with their maternal-effect sex determination system, which is the heterogametic sex? Explain briefly.

G. If a very young female human embryo were suddenly forced to chose between inactivating the paternal X in all her cells, or the maternal X in all her cells, which chromosome should she chose to inactivate? Explain.

H. An unusual species of bird is discovered for which sexual dimorphism in plumage color is determined by an "active W" for of sex determination, while sexual dimorphism in beak shape is determined by an "Z dose" system. If female plumage is normally red while male is blue, and female beak shape is long vs. male being short, what will the phenotype be of a rare ZZW; AA individual with respect to these two characters?
I. Recall that honeybees use a "multiple allele" system of sex determination. If an established beekeeper moved with his wife and young children to a dessert isle far out of range of other bees of the same species, and carried with him only a single hive (whose inhabitants were the progeny of a single queen who had mated to a single drone) -- when his sons and daughters grew up and took over the beekeeping chores, would they be likely to find more or fewer males produced by their hives than their father had told them had been the case in the "old country."

J. Recall that gynandromorphs are fruit fly genetic mosaics in which half the cells are XX and half are X0 and are caused by either the spontaneous loss (or the equivalent, failure to replicate) of an X chromosome during the very first division of the zygote nucleus. Recall the phenotype such mosaics was used to argue that the white gene is "cell autonomous," an important bit of information in deducing that both X chromosomes in fruit flies are simultaneously active in somatic cells.

(J-A) Do you think that X-chromosome dosage compensation itself is a cell-autonomous process? Explain.

(J-B) According to your answer to the first part of this question, describe the eye color(s) you would expect for the mosaic eye of gynandromorph derived from an XX zygote that was homozygous for the "leaky" white<sup>apricot</sup> allele that Muller used to deduce dosage compensation, given that half the eye in question is XX and the other half is XO. Explain.

K. There are diploid organisms that must mate to reproduce, but they do so in a rather peculiar way: females (attract and ) mate with males of a different species, since there are no males of their own species. The sperm from this different species serves only to activate the egg and does not contribute genes to the resulting embryo. Is this peculiar species having sex according to the definition you were given? Explain. Do you think this odd mating behavior is a newly acquired trait or something that has existed for a very long time (hint: there are parthenogenetic species that reproduce just fine without mating to any other organism).

L. If you were to be told that the marine worm Bonellia viridis has a 1:1 sex ratio, what additional (not unreasonable) feature of its unusual ESD mechanism of sex determination could you imagine that would reasonably account for such a ratio?

M. We talked about how Muller used the white<sup>apricot</sup> mutant allele to discover X-chromosome dosage compensation in fruit flies. In that same study, Muller studied the white<sup>eosi</sup>n, a hypomorphic mutant allele that behaved differently than white<sup>a</sup>. He found that white<sup>y</sup>/white<sup>a</sup> females had darker eyes than white<sup>y</sup>/Y males, but that white<sup>y</sup>/white<sup>a</sup> females had the same eye color as those males. It was later shown (with mutations that affect sexual differentiation only) that sexual phenotype per se has no effect on these eye colors.

(M-A) How do you think the eye color of a white<sup>y</sup>/Y; Dp(white<sup>a</sup>)/+ male (i.e. a male with two copies of the mutant allele) would compare to that of the other flies mentioned (namely, white<sup>y</sup>/white<sup>a</sup>, white<sup>y</sup>/Y, and white<sup>y</sup>/white<sup>a</sup>)? Explain briefly.

(M-B) If white<sup>y</sup>/Y males and white<sup>y</sup>/Y males have the same eye color, how do you think the eye color of white<sup>y</sup>/white<sup>a</sup> females would compare to that of white<sup>y</sup>/white<sup>a</sup> females? (darker,
lighter, the same? -- assume that the effects of each allele on eye color are strictly additive). Explain briefly.

**N.** If a recessive, somatic-cell-specific, cell-lethal mutation (i.e. a mutation eliminating the function of a gene needed for somatic cell growth and survival at all developmental stages) arose on the X chromosome during human spermatogenesis, and a sperm bearing this mutant allele fertilized an egg that carried a mutant allele of the X-linked *anhydrotic ectodermal dysplasia* gene (remember those heterozygous female twins with the patchy distribution of sweat glands?), how would the phenotype of the resulting daughter compare to that of a sister who was the product of a normal (non-mutant) sperm joining up with the same genotype of egg?
**ANSWERS**

Remember: it will be most useful for you to try to answer the questions on your own first, and only then look at the answers.

A:

Sensitization screens, since by that approach all the cells are mutant, so cell autonomy is not an issue. Mitotic recombination clonal screens require that the new mutation act cell autonomously in the clone itself to affect its phenotype.

B-A:

Genotype C only

B-B:

The $M^+$ parent, since upon recombination, the $M$ chromosome is lost -- you become homozygous for the $M^+$ chromosome which therefore is the one that is screened for mutations.

B-C:

A source of flp'ase and recessive marker mutations to tag the clones.

C-A:

Yes -- only two types of fertile matings can take place. In each case the mothers are $CyO/mel$, since $mel/mel$ moms are sterile. Matings with $mel/mel$ fathers will generate 50% $mel/mel$ sons, while matings with $CyO/mel$ fathers will generate only 33% $mel/mel$ sons. Hence, the fraction of males that are $mel/mel$ will be somewhere between these two values -- always in a minority.

C-B

No, not unless there is some viability difference between the sexes that has not been mentioned. All lethality mentioned should apply equally to both sexes.

C-C:

You want $mel/mel$ females. They can be recognized as the non-Curly (wildtype wing) females.

D-A:

This is clearly a ZZ/ZW system, the terminology we use when there are heteromorphic sex chromosomes and females are the heterogametic sex.

D-B:

Yes -- the fact that the female --ZW-- only carries a single allele of lgy indicates that genes on the W chromosome (including lgy) must have been lost.

D-C:

Haplo-insufficient genes.

D-D:

The fact that the single wildtype allele operating in $lgy^{12}/+$ males (9 legs) is less effective than the single wildtype allele in wildtype females (+/W -- 7 legs) indicates that a dosage compensation system must be operating to compensate for the lower dose of Z-linked alleles in females. Incidentally, the difference in phenotype between homozygous null males and hemizygous null females is irrelevant to this question (and in any event would argue that the female single + allele must be even more functional than one might otherwise imagine).

D-E:

It appears to be neomorphic, since the mutant phenotype is the same, whether one is increasing the dose of the mutant allele ($lgy^D/lgy^{12} = lgy^D/lgy^D$) or the
wildtype allele \((lgy^D/lgy^{12} = lgy^D/+).\) It would not be appropriate to consider it an antimorph just because the gain-of-function phenotype is the opposite of the loss-of-function.

D-F:

The conditions we call more "restrictive" when referring to a temperature-conditional mutant are those that generate a phenotype more unlike that of the wildtype. Hence in this case, the higher temperature, 30C, would be the more restrictive.

D-G:

Seven. An ideal temperature-conditional mutant is wildtype at the permissive temperature.

D-H:

A drop in leg number below 5 as we shift from 30C to 20C at progressively later points in development.

D-I:

Since \(lgy^{12}\) is an amorphic (null) allele, we can expect this cis arrangement of the two alleles to be functionally equivalent to \(lgy^{12}/+\), and hence have nine legs, regardless of temperature.

D-J:

As in D-I above, the two mutations in cis should be identical to the null allele by itself, so this genotype should have twelve legs -- same as \(lgy^{12}\) homozygotes, regardless of temperature.

D-K:

In each case, the two sexes would have the same level of \(lgy\) activity -- namely, none -- yet they display different mutant phenotypes, notwithstanding the fact that wildtype snargs exhibit no sexual dimorphism with respect to leg number. There must be some cryptic underlying difference between the sexes that is uncovered when \(lgy\) function is removed.

E:

Because the kind of mosaicism that is useful for F1 screening generates m/m clones in an m/+ background. In contrast, the F1 mosaics induced by EMS are a mixture of m/+ and +/+ cells, and hence are less than useless for revealing the phenotypes of newly generated recessive mutant alleles in the F1 generation.

F:

The female is the heterogametic sex, since it is her gametes (eggs) that carry the alternative forms (F and f) of the sex-determining gene that ultimately determine who will be male vs. female -- albeit one generation later than in most heterogametic systems.

G:

Since she is going to effectively become hemizygous for one X chromosome, she would be wise to make it the maternal X that she inactivates so that she can be sure that it is the paternal X that is active in all her cells. Since her father only had a single X, that X has already been tested for nasty mutations, while a particularly nasty mutant X chromosome could have been lurking in her mother’s genome. She would be wise to express the chromosome that has been tested most strenuously for deleterious recessive alleles.
H: Red plumage with short beak. Recall that in birds, the female (ZW) is the heterogamic sex (and if you don't recall what system birds use, at least recall that use of the ZW chromosome convention tells you the female is the heterogametic sex). Hence the "Active W" in our ZZW individual will impose the female plumage color character (red), while the "Z dose" will impose the male beak shape (short).

I: More. By founding a bee population with a hive generated by a single queen and a single drone, the bee population has only three different alleles at the sex-determining locus, in contrast to the large number normally found among an outbred bee population. Hence the likelihood of generating "diploid" males (homozygous for one of the three alleles) would be vastly increased -- and they would start appearing as soon as the present queen's daughter took over the next generation of queening chores.

J-A: Dosage compensation must be cell autonomous, since the XX and XO cells in a gynandromorph develop normally. Cell autonomy means that the cells' genotype dictates its phenotype with respect to the gene or process in question. If dosage compensation were not cell autonomous, then the rate of X-linked gene expression in XX cells might influence that in neighboring XO cells and cause those XO cells to express X-linked genes at a rate that is inappropriate for a cell with only a single X (and vice versa if the XO cells influenced the XX).

J-B: Since the white \textit{apricot} allele is sensitive to dosage compensation, and since the XX and XO cells in the mosaic eye are properly dosage compensating, the male and female halves of the eye will display the same (orange) color despite their different dose of the \textit{w} allele.

K: No -- there is no mixing of genes from two different individuals to generate new combinations of alleles. It seems likely that this is a fairly recently evolved behavior that allowed this species to become parthenogenetic without having to evolve a mechanism for activating their eggs by themselves. Since there is never any genetic contribution from the male to this whole process, it is difficult to imagine how the trait could persist for very long, since there would be nothing in it for the males (males learning to avoid such parasitic females would be more reproductively successful than those that did not). Moreover, since many species do manage to activate their own parthenogenetic eggs, clearly animals can evolve to do this -- and a female able to make that jump would seem to have an advantage over females that remain dependent on luring unrelated males.

L: Since females arise by sexually neutral larvae landing on rocks, while males are generated by the same larvae landing on females, you could get a 1:1 sex ratio if
any given female could only host a single larva whose metamorphosis it would shape (i.e. it would exclude other larvae once it had its male), and if larvae were sufficiently abundant so that nearly all females in the population acquired a larva to host.

M-A:

It appears that the \(w^b\) allele is not dosage compensated, so that the expression of the allele is the same in both sexes. Hence males with two copies of the allele will have the same eye color as females with two copies of the allele (\(w^b/w^b\)), and thus be darker than either \(w^b/Y\) males or \(w^b/w^-\) females.

M-B:

Because the allele is not dosage compensated, the contribution of a \(w^b\) allele to female eye color should be the same as it is in males, while the contribution of the dosage-compensated allele \(w^a\) should be half. Hence a \(w^b/w^a\) female should have lighter eyes than a \(w^b/w^b\) female.

M-C:

The effect of the cell-lethal mutation would be to cause cells that inactivated the mother's X chromosome (the chromosome carrying the only wildtype allele of this gene that is essential for cell growth -- but also carrying the defective \(ECD\) allele) to be selected against. Consequently only cells that had inactivated the father's X (the chromosome carrying the only wildtype \(ECD\) allele) would survive. As a consequence, the \(ECD\) mutant phenotype of this unfortunate daughter would be far more abnormal than that of her \(ECD/+\) sister who did not carry the paternally derived cell-lethal allele. Indeed, we would expect such a daughter to be as abnormal as her \(ECD/Y\) brothers.