Septoplasty by disarticulation

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Sex chromosome degeneration by regulatory evolution

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Summary

In many species, the Y (or W) sex chromosome is degenerate. Current theory proposes that this degeneration follows the arrest of recombination and results from the accumulation of deleterious mutations due to selective interference--the inefficacy of natural selection on non-recombining genomic regions. This theory requires very few assumptions, but does not robustly predict fast erosion of the Y (or W) in large populations or the stepwise degeneration of several small non-recombining strata. We propose a new mechanism for Y/W erosion that works over faster timescales, in large populations, and for small non-recombining regions (down to a single sex-linked gene). The mechanism is based on the instability and divergence of *cis*-regulatory sequences in non-recombining genome regions, which become selectively haploidized to mask deleterious mutations on coding sequences. This haploidization is asymmetric, because *cis*-regulators on the X cannot be silenced (otherwise there would be no expression in females). This process causes rapid Y/W degeneration and simultaneous evolution of dosage

compensation, provided that autosomal *trans*-regulatory sequences with sex-limited effects are available to compensate for *cis*-regulatory divergence. Although this "degeneration by regulatory evolution" does not require selective interference, both processes may act in concert to further accelerate Y degeneration.

Results and Discussions

The contemporary theory for the evolution of sex chromosome crystallized in the 1970s [1–3] and applies to both XX/XY and ZZ/ZW sex determination systems, which share important convergent similarities [4]. Both cases involve a chromosome that is heterozygous (the Y or W) and present in only one sex. Although a broad range of situations has been described, in many cases, most of the chromosome has stopped recombining and has degenerated considerably. Current theory, which we term "Degeneration by selective interference" (DSI) has been substantially refined since the 70s', but its core idea--degeneration caused by selective interference--has remained unchanged [3,5–8]. The theory about selective interference, as well as its empirical evaluation, has also been largely developed since the 70s', well beyond the case of Y degeneration [9–11]. DSI involves a sequence of steps that occur after the arrest of recombination between the Y and X chromosomes (all our arguments also apply to Z/W chromosome system): (a) degeneration of Y-linked genes by 'selective interference' (also known as the 'Hill-Robertson effect'), due to processes such as Muller's Ratchet, hitchhiking, and background selection [8], (b) facultatively, adaptive silencing of Y-linked genes, and (c) evolution of dosage compensation. A variant of this theory proposes that the accumulation of deleterious alleles in regulatory sequences by selective interference leads to reduced Y gene expression [12]. Y-linked alleles, being partially hidden would then further accumulate deleterious mutations and degenerate [13].

In this paper, we propose a new "Degeneration by Regulatory Evolution" (DRE) theory to explain Y chromosome degeneration. The main differences from the DSI model are that our theory does not require selective interference, and that steps a-c occur simultaneously after recombination suppression. We previously showed that, for autosomal genes, a '*cis*-regulator runaway' process occurs that leads stronger *cis*-regulators to become associated with

chromosomes with fewer deleterious mutations [14]. This favors the stronger *cis*-regulatory alleles, provided they are tightly linked to their coding gene. We also showed that *cis*-regulators diverge in asexuals, where diploid expression is unstable and quickly becomes "haploidized" [15]. DRE theory involves such divergence of *cis*-regulators, but with an asymmetry between the X and Y chromosomes (preventing the suppression of gene expression on the X). We investigate DRE using individual-based stochastic simulations of a population of N_{pop} diploid individuals, with *XY* males and *XX* females. In order to capture the essence of the mechanism, we first study a minimal system with only four loci (Figure 1): a gene *G*, its *cis*-regulator *C*, and two *trans*-regulators *T* (we will then extend the model to the case of a non-recombining region comprising a larger number of genes). *Trans*-regulators, such as transcription factors, are not closely linked to their target gene, and influence expression on both homologs, whereas c*is*-regulators, such as enhancers, control the expression of the closely linked gene, and influence only the copy carried on the same chromosome as themselves [16]. We assume that *G* and *C* are present on both sex chromosomes, and that they recombine (in females only) at a rate R_c. We include *trans*-regulators in order to examine whether, and over what time-scale, dosage compensation will evolve when expression of the Y-linked allele decreases. With these *trans*-regulators, overall expression can be maintained, i.e. dosage compensated, even if *cis*-regulators change and diverge between the X and Y. Dosage compensation cannot evolve if the *trans*-regulators act in the same way in both males and females, or equally on the X and the Y. Hence we do not consider all potential trans-regulators, but only those that could influence dosage compensation. Specifically, we focus on the simplest symmetrical case with one trans-regulator expressed in males T_m, and one in females T_f. Both cases have been described empirically (*C. elegans* dosage compensation works by halving X expression in females, whereas in *Drosophila*, it works by doubling X expression in males [17]). For simplicity, we assume that these *T* loci are autosomal and that they recombine freely with each other and with the *G* and *C* loci.

This initial model is later extended to n_L CGT_mT_f quadruplets of genes (where n_L = 1, 50, 500). In these models, we assume that the *C* and *G* loci are uniformly spaced on the sex chromosomes, with two adjacent genes *G* recombining at a rate R_g in females, and where each *C* locus is assumed to be closer to the *G* gene it regulates than to the next *G* gene (ten times

closer in the simulations,); again, recombination is assumed not to occur in males, representing a non-recombining region including the sex-determining locus, while all *T* factors are again assumed to recombine freely with the sex-determining region. In this model, each *CG* pair is influenced by its own T_mT_f pair, which represents the lowest degree of pleiotropy of these *trans*-acting factors, but involves a very high number of *trans*-regulators. We also considered a model where only one T_mT_f pair controls all the *G* and *C* loci, representing the other extreme case where *trans*-regulators are maximally pleiotropic and influence many (here all n_L) genes.

Deleterious mutations occur within genes *G* at a rate U_G per gene. Their fitness effect *s* is drawn from an exponential distribution with mean s_{mean}. The effects of multiple mutations in the same gene are assumed to be additive, but with a maximum effect per gene, s_{max} (which may be interpreted as the fitness effect of a full gene knock-out). Their dominance depends on the strength of their associated *cis*-regulator (see STAR methods). The effects of alleles at the *cis*- (C) and *trans*-regulators (T_m, T_f) are modelled as quantitative traits denoted by c, t_m, t_f, respectively, and control the level of expression *Q* of the gene, which is under stabilizing selection with intensity *I* (STAR methods). The different events of the life cycle occur in the following order: diploid selection, meiosis with recombination, mutation, and syngamy. Simulations are initialized with no polymorphism present, and the optimal gene expression level (no deleterious allele, all c and t_m, t_f alleles fixed to 1). After a burn-in phase where the chromosome evolves with recombination in both sexes, we stop XY recombination in males to create a sex-linked region and follow the frequencies and effects of deleterious mutations on the X and Y, as well as the evolution of the regulatory genes. These outputs are averaged over different numbers of replicates depending on the variance in the process under different parameter values (STAR methods, Table S1). At regular intervals, we compute $P_{halfsilent}$, the probability (across replicates) that Y allele-specific expression $\phi_{Y,i}$ decreased by two fold from the initial value of 0.5 to $\phi_{Y,i} < 0.25$. A complementary approach to quantify partial silencing is to measure the dominance coefficient of deleterious alleles on the Y, measured by $h_{Y,i} = \phi_{Y,i}{}^{-\ln h/\ln 2}$ (see STAR methods). We also compute P_{silent}, the probability that $\phi_{Y,i}$ becomes close to zero (below 0.01), so that alleles on the Y become nearly entirely recessive. The quantity $P_{halfdead}$ then refers to the probability that, by a given number of generations after the

Y-linked region stopped recombining, deleterious mutations on the Y gene copy have reduced fitness by an amount $s_{max}/2$, and P_{dead} that they reduced fitness by an amount s_{max}, indicating that the gene has entirely degenerated on the Y.

Figure 2 illustrates the process with n_L =1 (one gene, one *cis*-regulator, one male limited and one female limited *trans*-regulator). The system does not generate any male-female or X-Y asymmetry before recombination arrest (STAR methods, Figure S1). After recombination arrest, the gene carried by the Y degenerates: it becomes progressively recessive, as h_Y changes from *h* = 0.25 to zero, and accumulates deleterious mutations (the overall fitness effect of mutations present on the Y copy increases up to s_{max}), despite there being very limited selective interference (at most only occurring between the gene and its *cis*-regulator). Silencing occurs first, and the accumulation of deleterious mutations follows later in the process (the curve representing $P_{halfsilent}$ is ahead of the one showing the accumulation of deleterious mutation on the Y, as measured by $P_{halfdead}$). Degeneration also occurs with full dosage compensation, and overall expression never departs from the optimum in either sex (Figure 2B). Compensation typically involves, at least initially, a mixture of upregulation of X gene copies in males and downregulation in females (STAR methods, Figure S3).

What is the underlying cause of this asymmetrical degeneration? Once X and Y stop recombining, diploid expression becomes unstable. *Cis*-regulators on the X and Y can diverge, eventually leading to the haploidization of expression in males. This is not prevented by stabilizing selection on expression levels as long as *trans*-regulators can coevolve to maintain near optimal total expression in both sexes. When the strength of *cis*-regulators on the Y starts decreasing, the process is accelerated by a "haploidization" positive feedback loop. Indeed, weak Y *cis*-regulators become associated with coding sequences carrying more deleterious mutations, as they cause a reduction in dominance. They are then selected to weaken further in order to mask those deleterious mutations, which leads to the accumulation of even more deleterious mutations, and so forth. By contrast, the other 'haploidized' situation (where the X is silenced in males) is reversible, as X chromosomes with weak *cis*-regulators and a higher load of deleterious mutations cannot fix, as they become homozygous and selected against in females when too frequent (unlike partially silenced Y genes, which can spread as they stay

heterozygous in males). Therefore, the regulatory system has only one stable equilibrium, where the Y is silenced and degenerate.

Selective interference plays no role in this process, explaining why degeneration occurs even for a single Y-linked gene. However, the process is stochastic, as it is initiated by a random departure from diploid expression with a sufficiently weak Y *cis*-regulator to trigger the "haploidization" feedback loop. In individual simulation replicates, degeneration is indeed very abrupt, but occurs at varying time points (Figure 2A). Because of this stochasticity, the process is slowed down in larger populations: it is ∼10 times slower in ∼10 times larger populations (Figure 2D). With the same parameters but without mutation in the *cis*-regulator (U_c = 0, Figure 2C), as in DSI theory, degeneration does not occur, as expected, since there is neither selective interference nor *cis*-regulatory divergence. Degeneration does not occur either in the absence of mutation in the *trans*-regulators (U_t = 0, Figure 2C) and for the same parameter values, since *cis* and *trans*-regulators have to coevolve to maintain total expression levels: if *trans*-regulators cannot evolve, the divergence of *cis*-regulators is prevented and Y degeneration cannot occur. However, if the intensity of stabilizing selection on expression levels is weak enough, degeneration evolves but is not dosage compensated (Figure 2C). Otherwise, the intensity of stabilizing selection on dosage only plays a marginal role in DRE (Figure 2C). Control simulations without mutation in the coding gene (U_G = 0, Figure 2C) show that *cis*-regulatory divergence and Y silencing can occur even in the absence of deleterious mutations, but as expected, this silencing is slower, not being accelerated by the "haploidization" feedback loop, and reversible (STAR methods, Figure S2).

As expected, DRE and DSI combine when more than a single Y-linked gene is considered. The effect is strong: Figure 3 shows that a 50-fold or 500-fold increase in the number of loci results in degeneration being 5-fold and 10-fold faster, respectively: a larger non-recombining Y-linked region degenerates faster than a small one. Without mutation in regulators (i.e. with only DSI), degeneration occurs but is 23-36 fold slower with 50 loci (depending on the control used for the comparison, STAR methods, Figure 4). With 500 loci, however, the comparison with and without regulators is problematic, as very quickly, a modest accumulation of deleterious mutations on the many Y-linked genes causes an important reduction in male average fitness, so that male

fitness reaches unrealistically low values (of the order 10^{-17}). Even if a proportion of genes affecting male fitness may be under soft selection, it seems unlikely that a population with such a low male fitness would survive.

The drop in male fitness is less dramatic with regulatory evolution, as those mutations become progressively more recessive as the Y degenerates. There is nevertheless a transient drop in male average fitness, which can be quite large (e.g. 3% and 85% reduction for a 50-gene and 500-gene Y-linked region, respectively, Figure 3). Data on divergence between sex chromosomes indicate that Y degeneration is often sequential in chiasmate species, with several regions of various sizes, termed "strata" [18], having stopped recombining at different time points. This high transitory fitness drop may prevent large strata from occurring in small populations and may bias towards scenarios involving multiple small strata as, for example, in humans [19]. Comparatively, these scenarios involving small strata are more difficult to explain with DSI, as selective interference is weak on small non-recombining regions.

Last, degeneration is initially slower but faster overall when there is only one T_m and T_f controlling all *cis*-regulators. Despite being highly pleiotropically constrained, having only two autosomal *trans*-regulators precipitates degeneration: the tipping point where it is worth fully silencing the Y is quickly reached when many very weakly deleterious mutations have accumulated on the Y. This is consistent with the observation that dosage compensation can occur locally on a gene-by-gene basis or by chromosome-wide *trans*-acting effects [17]. Intermediate cases involving *cis*- and *trans*-regulators with regional effects [as e.g. in Drosophila 20] may be worth investigating, but are likely to behave similarly, as long as *trans*-regulators only target X genes that have a copy on the Y non-recombining region.

Simulations for lower values of mutation rates and strength of selection against deleterious alleles are shown on Figure S4 (for n_L = 1 and n_L = 50, STAR methods). Unsurprisingly, reducing mutation rates slows degeneration, while the effect of s is more complicated. However, the acceleration of degeneration caused by regulatory evolution still holds. Figure S4 also shows that a scaling argument from diffusion theory indicates that larger populations with weaker mutation and selection should also behave similarly, albeit on a longer timescale.

198 The DRE theory proposes a different view of sex-chromosome evolution compared to the DSI
199 theory that has been developed over the past 40 years. In both cases, degeneration starts after
200 the arrest of recombination in a genome region completely linked to the sex-determining locus.
201 In both cases, degeneration is slower in larger populations, but this is considerably less so in the
202 DRE model. However, there are important differences. With very few exceptions [21,22], DSI
203 was developed without explicitly modelling regulatory evolution. With DSI, regulatory evolution
204 (Y silencing and dosage compensation) is supposed to occur only after deleterious mutations
205 have accumulated on the Y [3,23–25], although it has also been proposed that silencing may
206 result directly from the accumulation of deleterious mutations in regulatory regions [13]. This is
207 certainly possible, as selective interference applies to all functional sequences and may
208 contribute to the fixation of many kinds of deleterious mutations, including those maintaining
209 adequate expression levels. DRE is based on a reverse causality: regulatory evolution initiates
210 the degeneration process. Contrary to the standard model where compensation is needed
211 because degeneration damages genes' function in males, compensation evolves here from the
212 very beginning of the process, by reducing the proportion of transcripts from Y-linked relative to
213 X-linked alleles and maintaining an almost constant overall level of expression in both sexes.
214 However, compensation may not occur when expression levels are under weak stabilizing
215 selection (Figure 2C). Whether compensation occurs on a gene-by-gene basis or chromosome
216 wide depends on the availability of the corresponding *trans*-acting factor, but both can occur in
217 DRE, and, surprisingly, at approximately the same rate (Figure 3).

218 The specific mode of dosage compensation depends on the type of *trans*-regulators. Here we
219 considered *trans*-regulators with sex-limited expression, which can mimic several well-known
220 dosage compensation systems (female-limited factors corresponding to *C. elegans* or mammal
221 systems, while male-limited factors would be more similar to a *Drosophila*-like situation [17],
222 STAR methods). The symmetric DRE model that we used often led to a Drosophila-like
223 compensation, where the X in males was eventually expressed twice as much compared to the
224 situation at the recombination arrest (Figure 2B). However, this is certainly dependent on the
225 relative mutation rates on the different types of *trans*-regulators. The theory should be

extended to examine the diversity of dosage compensation mechanisms, including sex-of-origin effects [26].

Overall, we have presented an alternative theory for the degeneration of sex chromosomes. Although many underlying parameters are still poorly known, this theory could be tested quantitatively, as it works faster than current theory, on smaller non-recombining regions, and does not require small population sizes or recurrent beneficial mutations causing hitchhiking effects. It does not exclude selective interference, which will necessarily co-occur as long as many genes stop recombining simultaneously. However, a hallmark of DRE is that regulatory changes occur very early. This is consistent with recent studies showing that dosage compensation evolves early on [27,28] and that Y transcriptional downregulation accompanies degeneration of protein-coding genes from the start [29–31].

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Author contributions

Original idea TL, FF; Model conception TL, DR, FF; Code DR, TL, ES; Simulations TL, ES; Data analyses TL; Interpretation TL, DR; First draft TL, FF; Editing TL, DR, ES; Reviews TL, DR; Project management and funding TL.

Declaration of interest

The authors declare no competing interests.

Figure Legends

Figure 1 Model presentation. (**A**) The simplest genetic model involves autosomal *trans*-regulators expressed either in males or females, and a sex-linked *cis*-regulator controlling the expression of a coding gene. A deleterious allele **a** at the coding gene is expressed at the same level as the wild type **A** allele if their associated *cis*-regulators have equal strength. (**B**) If there is *cis*-regulatory variation, the deleterious allele may be over or under-expressed, depending on whether it is associated with the stronger or weaker *cis*-regulator. This can be considered as determining the dominance of the **a** allele. The black curve shows the fitness of an Aa heterozygote (y-axis) for varying allele-specific expression levels (x-axis). (**C**) Selection also acts on the total amount of protein produced (x-axis), with stabilizing selection around an optimal amount. The maximal fitness effect of a departure from the optimal amount is s_{max}, the same as the maximal fitness effect of a deleterious mutation in the coding gene.

Figure 2. Y degeneration by regulatory evolution. (A) x-axis: time in number of generations, in log-scale. Recombination stops between the X and Y chromosome at generation 250000 (vertical dashed gray line); y-axis: The probability that a coding gene on the Y is expressed at less than half the level of the X copy (half-silent, light green curve), entirely silenced (dark green curve), has accumulated deleterious mutations reducing fitness effect half as much as a loss-of-function mutation (half-dead, orange curve), or as much as a full loss-of-function mutation (dead, red curve). Curves are averages over 100 replicates of the process. The model was run with n_L = 1 (one gene, one *cis*-regulator, one female and one male limited *trans*-regulator). Parameter values are: population size $N = 10^5$; Mutation rates U_G, $U_C = 2 \times 10^{-4}$; $U_T = 10^{-4}$; recombination rate between the gene and the *cis*-regulator $R_C = 5 \times 10^{-5}$ (in both sexes during the burn-in period, and in females thereafter). The mean effect of each deleterious mutation in the coding gene s_{mean} = 0.05 (and loss-of-function effect s_{max} = 0.3, dashed gray line). The dominance coefficient of deleterious mutations (when both alleles are equally expressed) is h = 0.25, and the intensity of stabilizing selection on dosage I = 0.1. The figure also shows the dominance of deleterious mutations carried on the Y (h_Y, blue curve) and the average fitness effect of alleles on the Y (s_Y, purple curve). Some individual trajectories are indicated for h_Y and s_Y (same color code, thin lines) to show that degeneration occurs abruptly in each replicate. **(B)**

Time-variation of regulatory traits corresponding to the case illustrated in panel a. x-axis: time in number of generations (in log-scale); y-axis: regulator trait values. Pink: X *cis*-regulator strength; Blue: Y *cis*-regulator strength; Brown: *trans*-regulator strength (plain: female limited, dashed: male-limited). Optimal dosage is 2 (dashed gray line). Total expression in males and females is indicated by the dotted curves (male value in blue, female in red). Regulatory trait values before recombination arrest are not stable due to runaway evolution [14], but are rescaled at 1 at generation 250000 for fair comparisons across parameter values. They are represented at this rescaled value on the figure, to avoid overloading the figure (see STAR methods and Figure S1-S3 for further details). **(C)** Values of s_Y, h_Y and P_{dead} at generation 3 x 10^6, for simulations like in panel (a) except for some parameter values. The star indicates weaker stabilizing selection (I =0.01) compared to I = 0.1 in panel (a). NOG (U_G = 0); REC (no arrest of recombination); NOCT (U_C = U_T = 0); NOT (U_T = 0); REG (with mutations on gene and regulators, as in panel a); FREE (R_c = 0.5); SYM (symmetrized stabilizing selection function, see STAR methods). **(D)** Accumulation of deleterious mutations on the Y for different population sizes (10^3, 10^4 like in panel a, 10^5). *x*-axis: number of generations in log-scale. *y*-axis: s_Y. Plain lines: with evolving regulators. Dotted lines: without regulator evolution (NOCT simulations, U_C = U_T = 0). Table S1 summarizes the different simulations, parameter values and number of replicates.

Figure 3 DRE with many loci. Y degeneration in non-recombining regions with n_L loci. **(A)** n_L = 50. **(B)** n_L = 500. Coding genes are positioned at regular interval on the X/Y chromosome, each at a recombination rate R_G = 5 x 10^{-4} (map length of the non-recombining region is therefore 2.5 or 25 cM, respectively). Recombination occurs in both sexes during the burn-in period (ending at 2.5 x 10^5 generations, vertical dotted line), and in females thereafter. *x*-axis: number of generation in log-scale. *y*-axis: degeneration of the Y, as measured by P_{dead}, in log-scale (plain lines) or male mean fitness (dashed lines). Red: the n_L = 1 case given for comparison. Dark red: n_L genes with n_L male-limited *trans*-acting factors and n_L female-limited trans acting factors. Brown: n_L genes with 1 male-limited *trans*-acting factor and 1 female-limited trans acting factor influencing all *cis*-regulators. Gray: n_L genes without regulatory evolution (NOCT simulations, U_C = U_T = 0). Other parameters are as in Figure 2A. Note the different time range in panels (a) and (b). In the absence of regulatory evolution (NOCT simulations), with n_L = 500, male average

fitness drops quickly to a vanishingly small number (to the order 10^{-17} after a million generation) that should lead to population extinction, as indicated by the skull symbol. This drop occurs quickly, even before any appreciable mutation accumulation on genes (i.e. it is already down to the inverse of population size when s_Y reaches ~0.01). Table S1 summarizes the different simulations, parameter values and number of replicates. See also Figure S4.

Figure 4 Comparing DRE and DSI. Y degeneration in non-recombining regions with 50 loci. Coding genes are positioned at regular interval on the X/Y chromosome, each at a recombination rate R_G = 5 x 10^{-4} (map length of the non-recombining region is therefore 2.5 cM). Recombination occurs in both sexes during the burn-in period (ending at 2.5 x 10^5 generations, vertical dotted line), and in females thereafter. *x*-axis: number of generations in log-scale. *y*-axis: degeneration of the Y, as measured by P_{dead}, in log-scale. **Dark red**: 50 genes, with 50 *cis*-regulators, 50 male-limited *trans*-acting factors and 50 female-limited trans acting factors. **Dark red, dashed**: 50 genes, with 50 *cis*-regulators, no mutation in *trans*-regulators (NOT simulations, U_T = 0). **Gray, dashed**: 50 genes, with 50 *cis*-regulators, no *trans*-regulators (NOT simulations, U_T = 0), but dominance of the effect of mutations on genes maintained at h = 0.25. **Gray**: 50 genes without regulatory evolution (NOCT simulations, U_C = U_T = 0). Other parameters are as in Figure 2A. Table S1 summarizes the different simulations, parameter values and number of replicates. See also Figure S4.

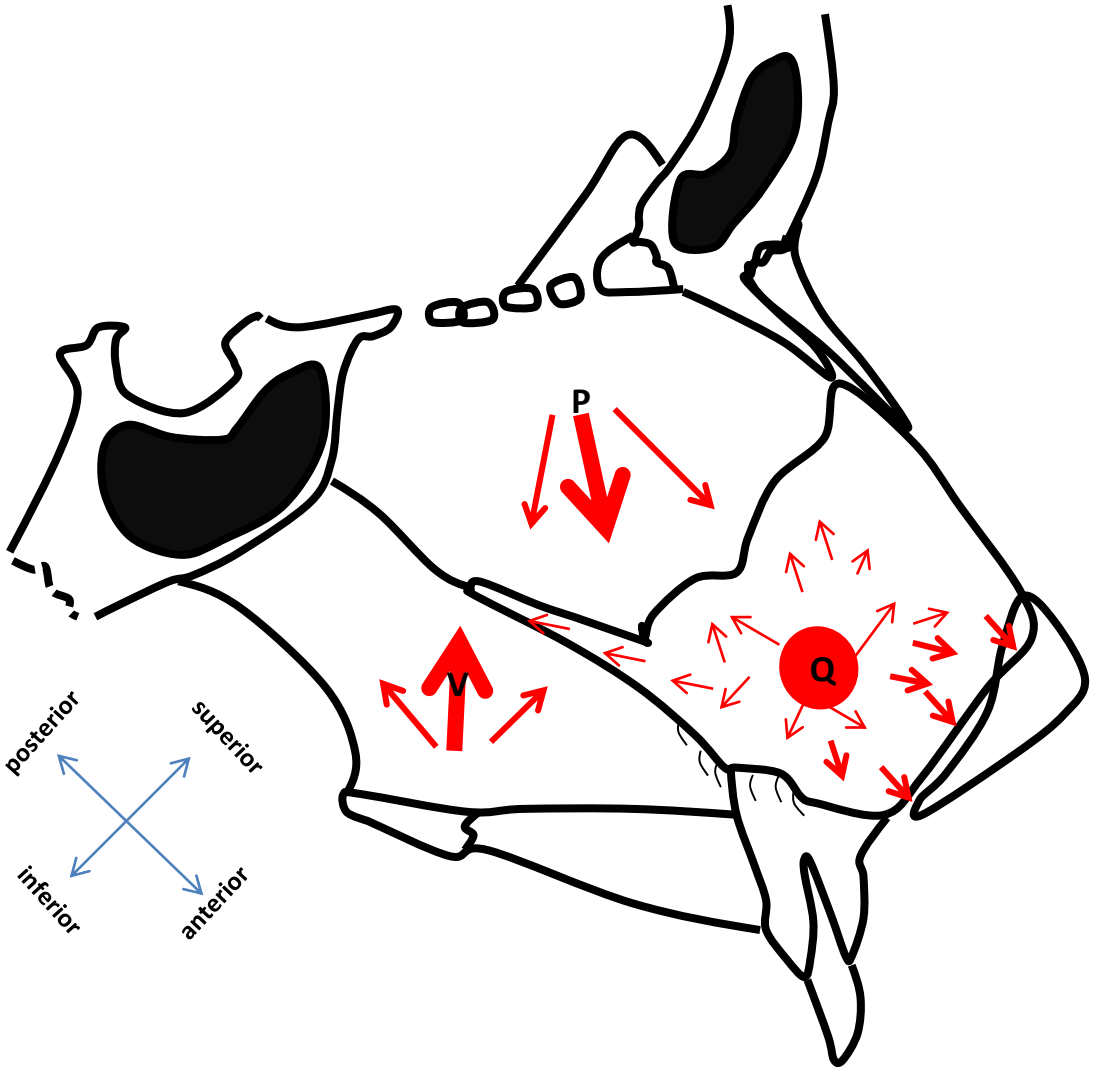
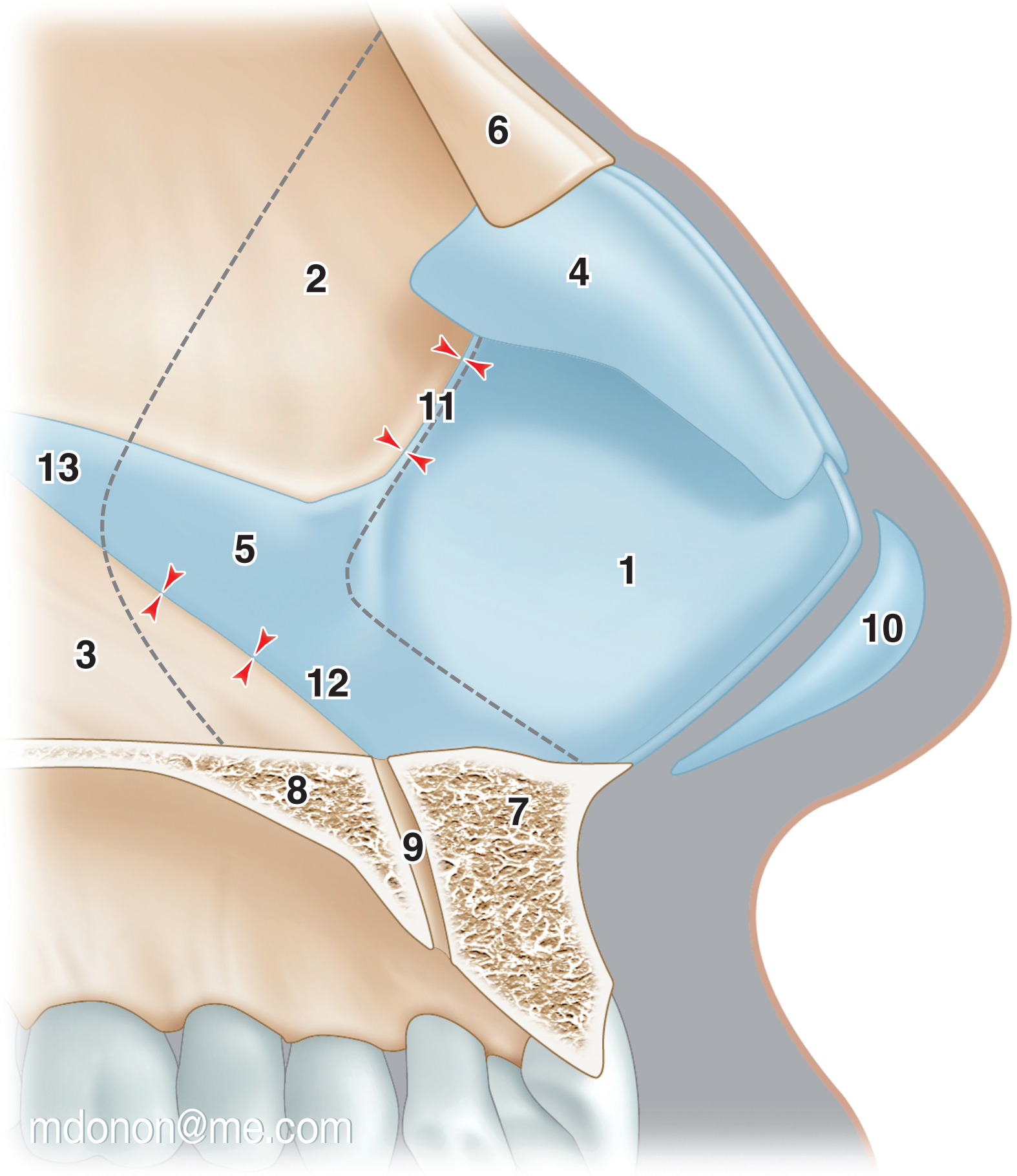
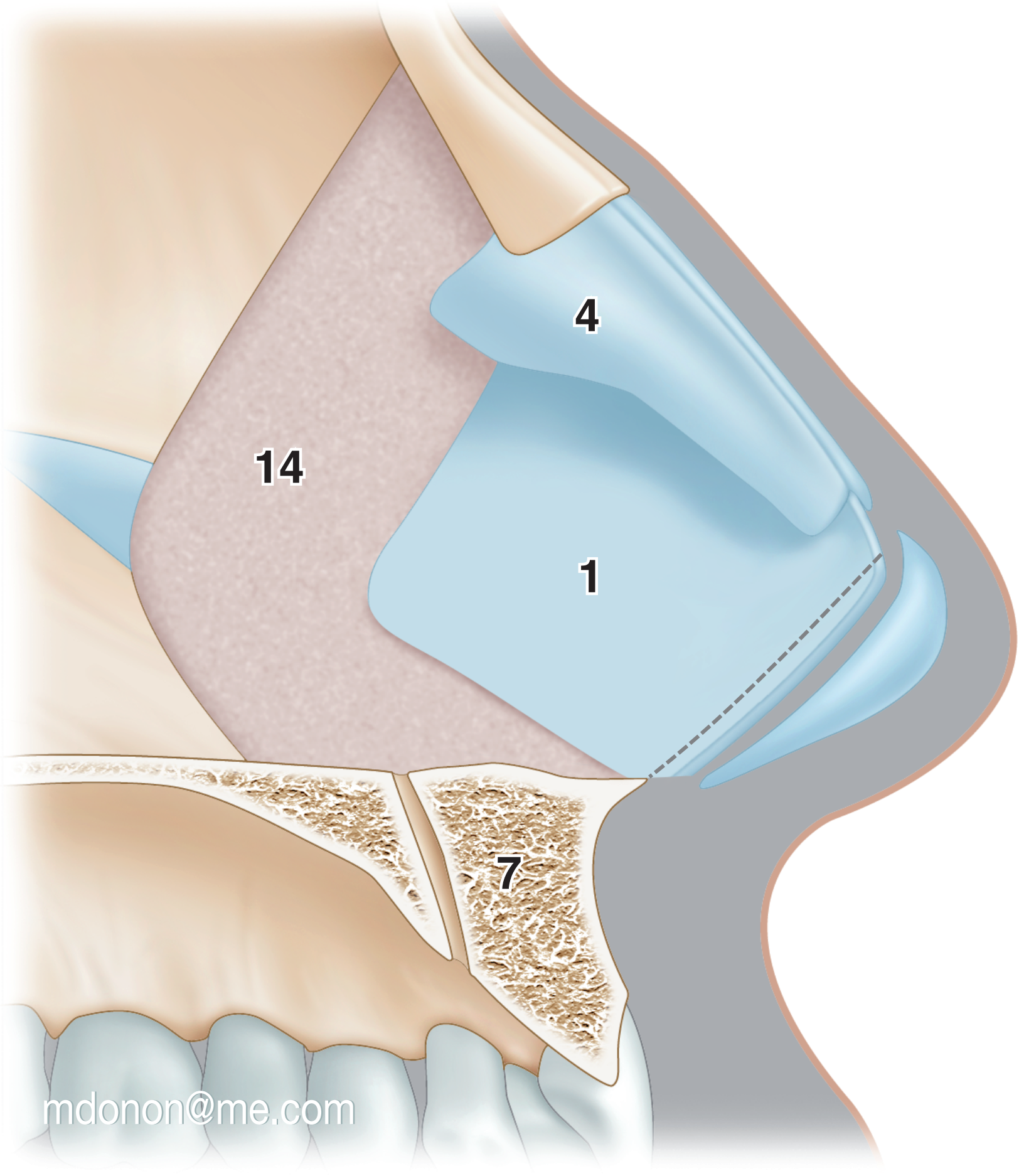


Figure 1





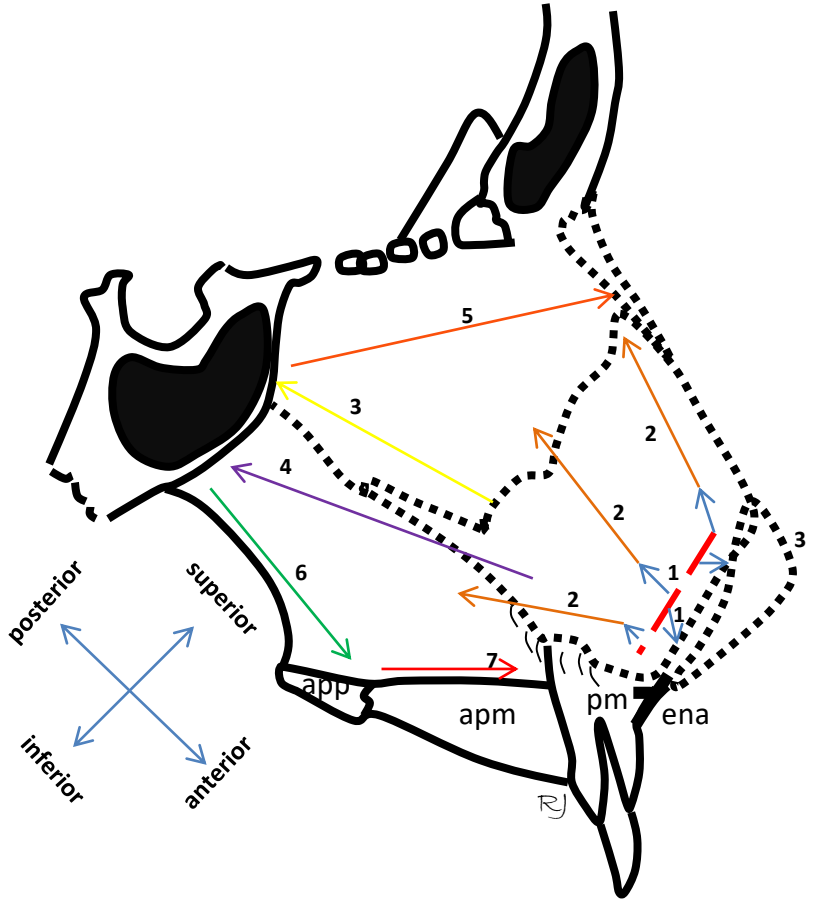

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Figure 4

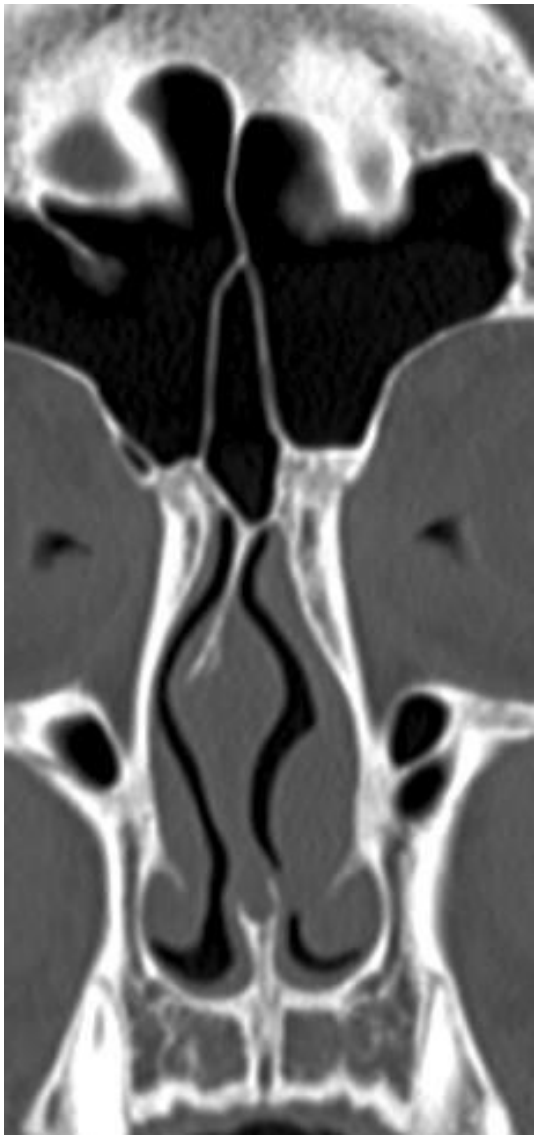

Figure 5: