

Evolution de l'appareil urogénital et de la détermination du sexe

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DES SCIENCES
D'ORSAY

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Evolution

- Evolution des organes – Evo-Dévo
- Evolution des traits d'histoire de vie
- Evolution chromosomique
- Evolution de caractères sur une phylogénie

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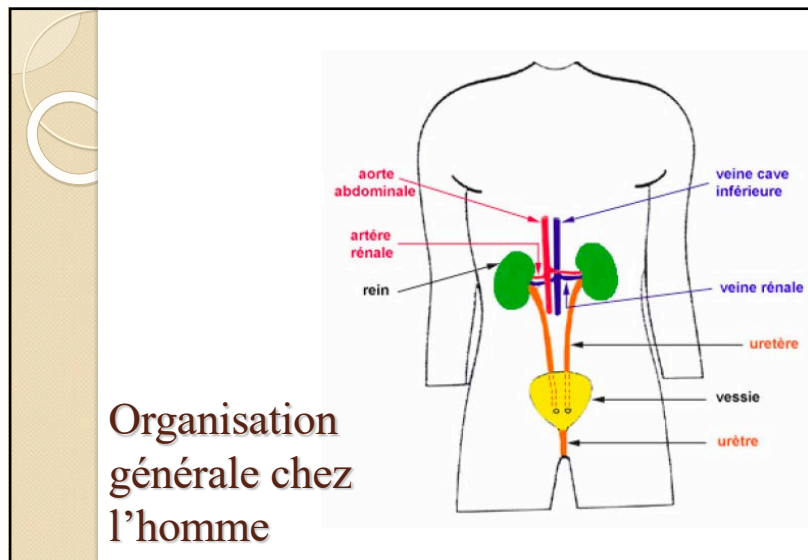
L'APPAREIL EXCRÉTEUR

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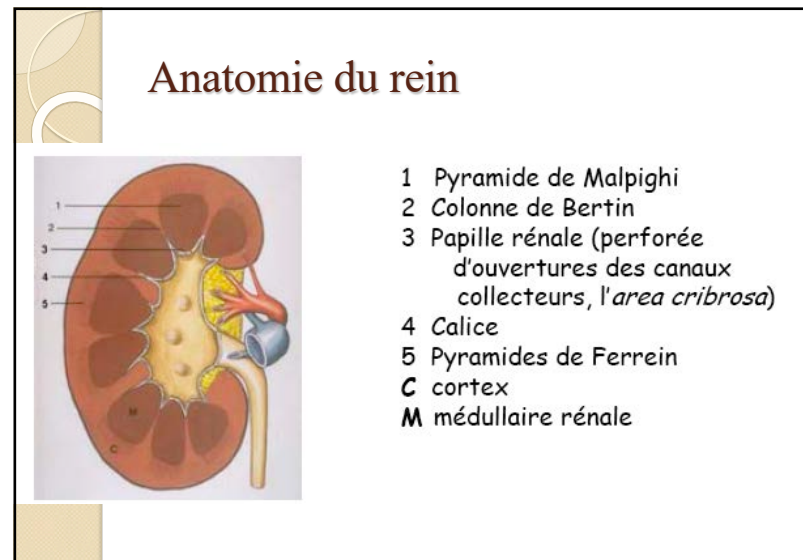
L'appareil excréteur

- Deux reins pourvus chacun d'un conduit évacuateur qui débouche dans le cloaque (au moins chez l'embryon).
- Chez le mâle des gnathostomes (sauf téléostéens), des liaisons existent entre la gonade et le système excréteur qui assurent l'évacuation du sperme.

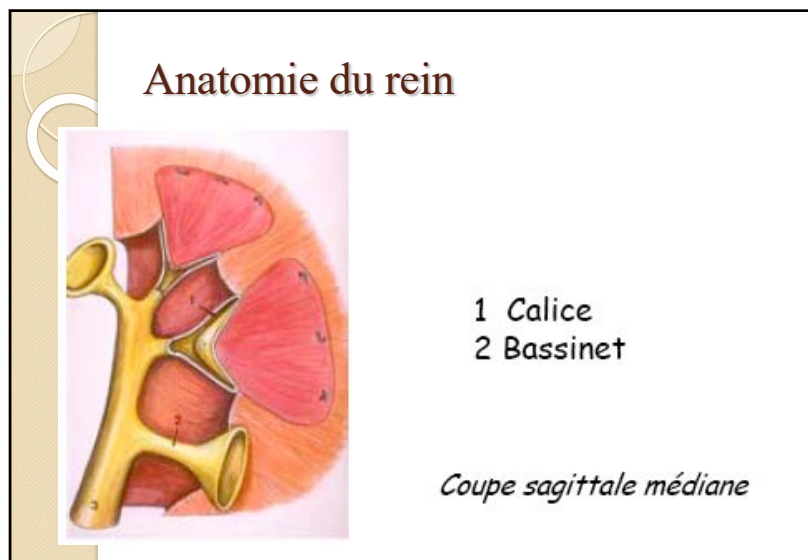
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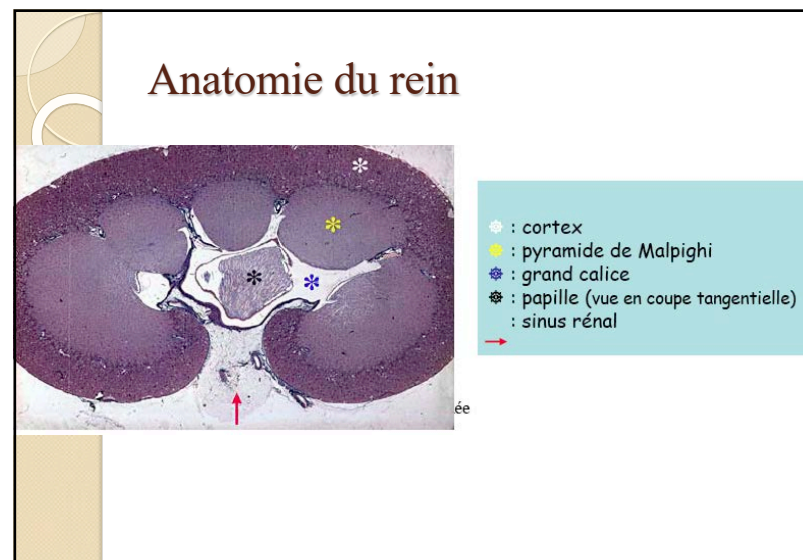
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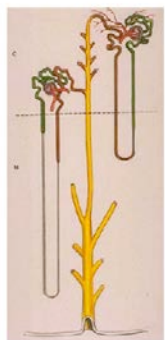
Le néphron

Unité fonctionnelle du rein

- 2 à 5 cm de long
- 1 à 1,4 million par rein (3 fois les besoins)
- vascularisée et innervée

constituée

- d'un **corpuscule rénal, de Malpighi**
touffe de capillaires, situé dans le cortex (C), assurant la *filtration du sang*
- d'un **tube urinaire**, succession de tubules, dans le cortex (C) et la zone médullaire (M) assurant la *réabsorption et la sécrétion*
 - le **tube contourné proximal TCP** (vert)
 - l'**anse grêle de Henlé** (grise)
 - le **tube contourné distal TCD** (brun)



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Type de néphron en fonction de la localisation du glomérule

- **Néphrons corticaux superficiels**,
- **Néphrons corticaux profonds**, situés dans les pyramides de Ferrein, courte anse grêle de Henlé
- **Néphrons juxtamédullaires** plongeant dans les pyramides de Malpighi, anse grêle de Henlé très longue

(c'est la longueur de la portion grêle de l'anse de Henlé qui permet d'établir un gradient de concentration dans la médullaire rénale)

Des canaux collecteurs (jaune) ou tubes de Bellini acheminent l'urine formée par le tube urinaire vers les voies excrétrices.

La boucle de Henle a été nommée d'après Friedrich Gustav Jakob Henle (1809-1885) qui la décrit vers 1860.



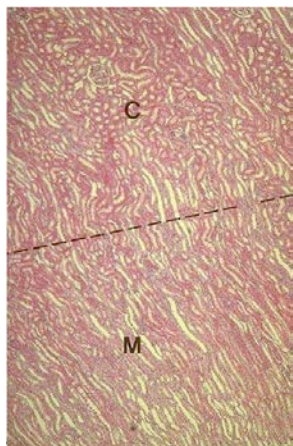
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Coupe à faible grossissement

Coupe de rein à faible grossissement

Cortex : corpuscules
larges tubes colorés,
TCP et TCD et TC

Médullaire :
anses de Henlé
larges tubes à grande lumière,
les tubes collecteurs TC



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Le corpuscule de Malpighi

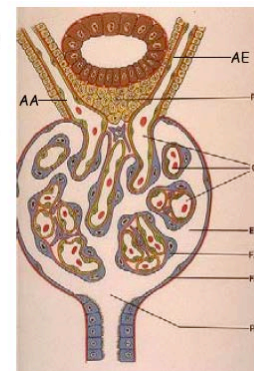
Glomérule : une artériole afférente (AA) donne naissance au niveau du pôle vasculaire (PV) à un peloton d'anses capillaires C drainé par une artériole efférente (AE)


Capsule de Bowman (capsule épithéliale à double paroi):

- le **feuillet pariétal (FP)** ou épithélium capsulaire tapisse la LB et délimite le corpuscule de Malpighi
- le **feuillet viscéral (FV)** ou épithélium glomérulaire recouvre l'ensemble des anses capillaires, il est constitué de cellules appelées **podocytes**

Chambre glomérulaire (EB) : espace compris entre les deux feuillets de la capsule de Bowman

Au pôle opposé ou **pôle urinaire (PU)** l'épithélium du feuillet pariétal de la capsule de Bowman se transforme en celui du tube contourné proximal TCP qui achemine le filtrat glomérulaire.



 William Bowman (1812-1891) comprend le lien entre le glomérule et les tubules, décrit les cellules épithéliales pariétales, la membrane basale et (avec Robert Todd) identifie les cellules endothéliales.

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Marcello Malpighi



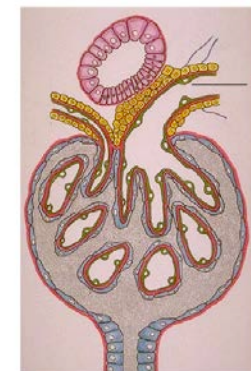
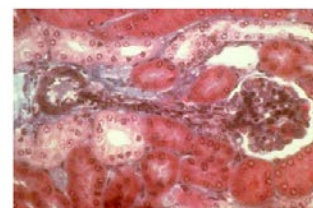
- Médecin et anatomiste italien
(Crevalcore, 1628 - Rome, 1694)
- A cette époque, les vues de Malpighi provoquent controverses et jalousie car il refuse de suivre aveuglement les théories de "maître" Galien et doit affronter un environnement hostile.
- Il travaille à expliciter les constituants microscopiques de divers organes comme le foie, la rate, les reins et les os. Différents insectes passent également sous la lunette de son microscope .

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Le glomérule

L'artériole afférente AA du glomérule

- plus large que l'artériole efférente maintient le gradient de pression nécessaire à la filtration
- disparition de la LEI
- certaines cellules musculaires lisses de la média entrent dans la constitution de l'appareil juxta glomérulaire.



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Antoine Ferrein

- Antoine Ferrein essaye de démontrer l'absence des corpuscules de Malpighi en 1749 en proposant que ceux-ci ne sont que la fin du tubule. Il laissera quand même son nom aux pyramides...

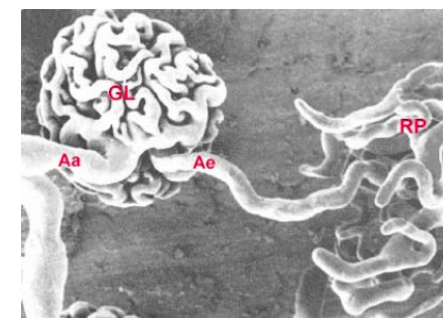


Antoine Ferrein, né à Frespech (Lot-et-Garonne) le 25 octobre 1693 et mort à Paris le 28 février 1769

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Microscopie électronique

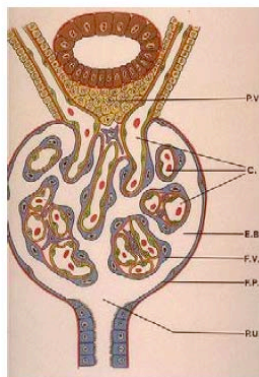
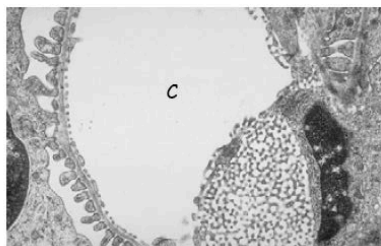
Microscopie électronique à balayage, après injection de résine: mise en évidence de l'artériole efférente (Ae) qui poursuit son trajet en dessinant un riche réseau vasculaire péri-tubulaire (RP) (x400)



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Endothélium des capillaires du glomérule

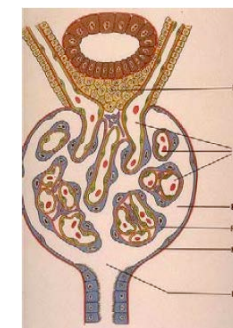
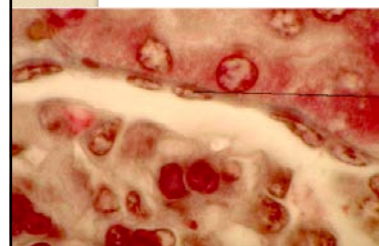
cellules endothéliales fenêtrées
pores de 50 à 100nm de diamètre
glycocalix anionique enjambant les pores
- pas de passage des cellules du sang
- ni de macromolécules comme l'albumine



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Capsule de Bowman

Feuillet pariétal (FP) épithélium simple pavimenteux, se poursuit au *pôle vasculaire* en *feuillet viscéral (FV)*, devient prismatique au *pôle urinaire*, paroi du TCP.



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Feuillet vicéral MEB x5500



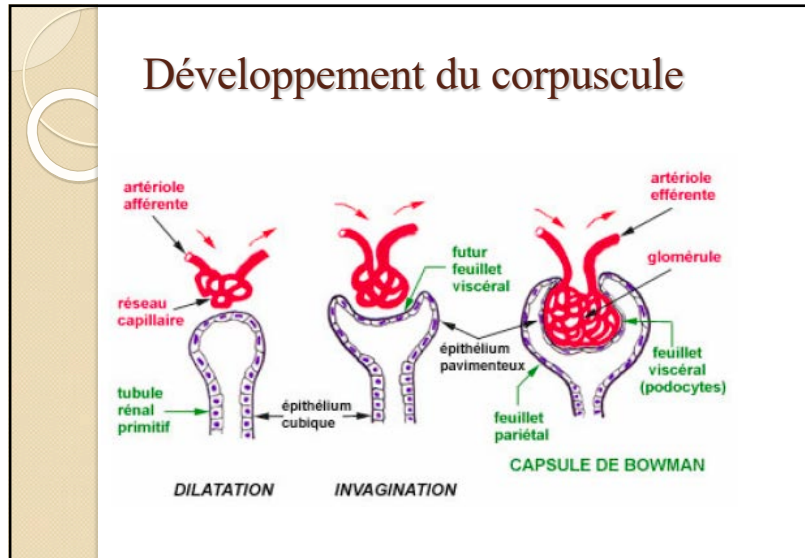
Constitué de **podocytes (CC)**, cellules à longues expansions primaires PP (majeures) et secondaires ou **pedicelles Pé** entrelacés avec ceux des podocytes voisins, ménageant des **fentes interpodocytaires** (25nm), pedicelles réunis par une *mince membrane* (5nm) parallèle à la LB des capillaires (ou **diaphragme**).

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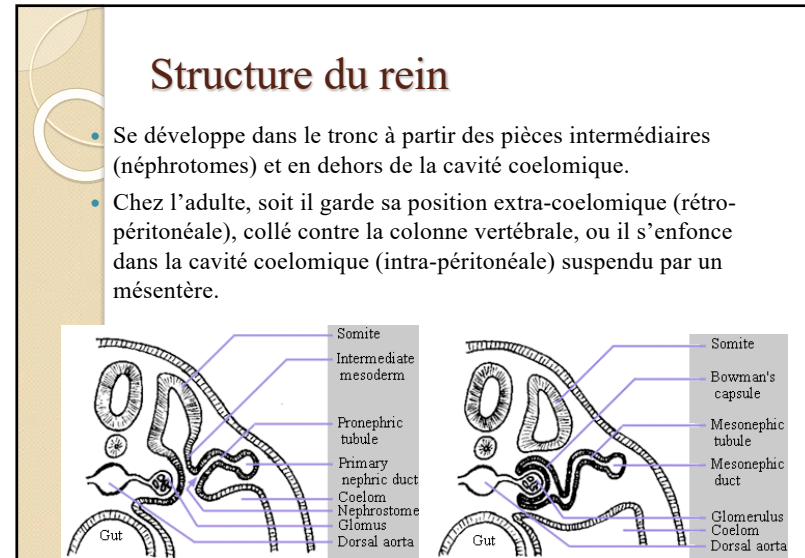
Histologie



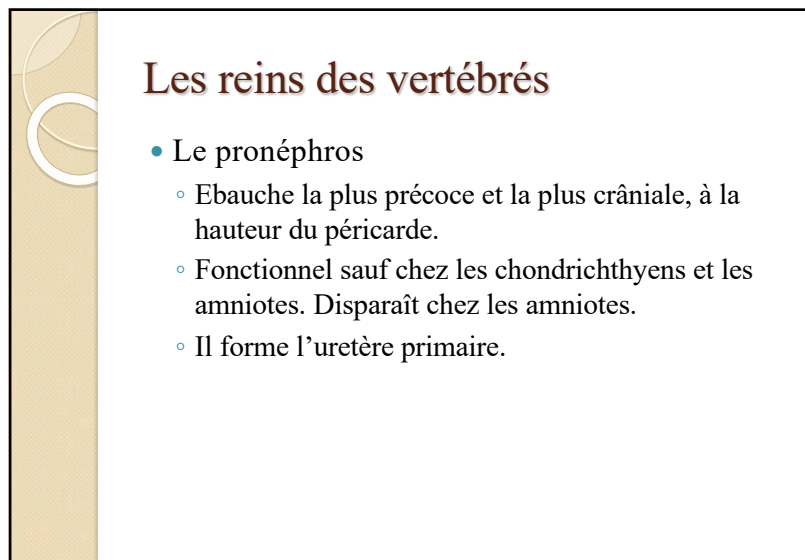
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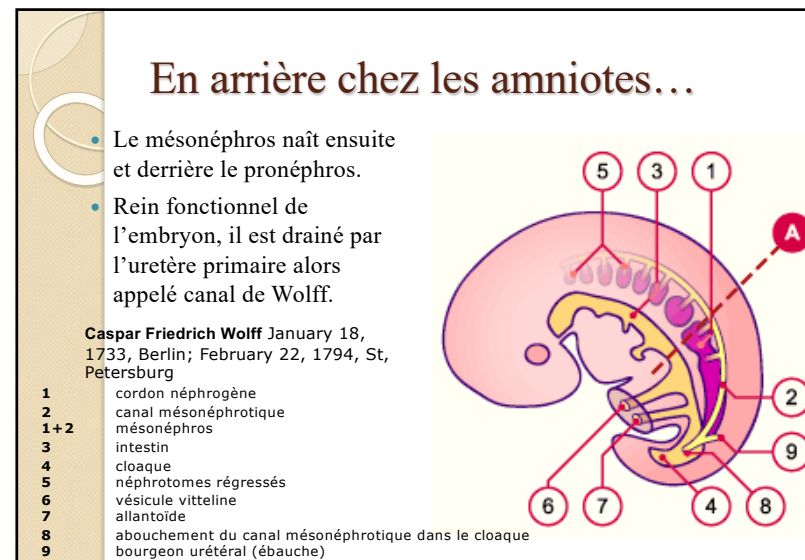
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Croissance du canal de Wolff

- L'expérimentation a permis de montrer que la croissance et la migration caudale du canal mésonéphrotique est due à la présence de molécules dans la matrice extracellulaire, notamment une intégrine, la fibronectine. La différenciation du cordon plein en un canal est quant à elle subordonnée à la sécrétion de BMP4 (bone morphogenetic protein 4) par l'ectoderme de surface.

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Le métanéphros

- Deviendra le rein adulte, le plus tardif et le plus postérieur.
- Drainage par des uretères secondaires qui bourgeonnent à partir de l'uretère primaire.

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L'évacuation

- L'uretère primaire est édifiée par le pronéphros. Au début est seulement un conduit excréteur.
- L'établissement d'une liaison avec le testicule lui confère ensuite un double rôle, excréteur et génital (uro-spermiducte).
- Chez de nombreux groupes, en particulier les amniotes, l'uretère est annexé par la gonade et devient un canal déférent.
- L'uretère primaire peut bourgeonner des uretères secondaires qui le remplace comme vecteur d'urine.
- Chez la femelle, n'a jamais de rôle génital.

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° L'APPAREIL GÉNITAL

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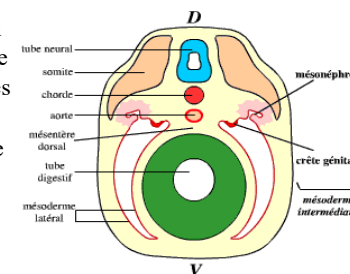
Origine de la gonade

- Elle naît d'une crête génitale occupant presque toute la longueur du coelome mais dont seule la partie moyenne, gonale, se différencie en glande fonctionnelle.
- Chez les apodes et les urodèles, le reste de la bande subsiste sous la forme d'une bandelette graisseuse dont l'ablation provoque la dégénérescence des gonades.
- La partie postérieure, épigonale, subsiste aussi chez beaucoup de sélaciens.

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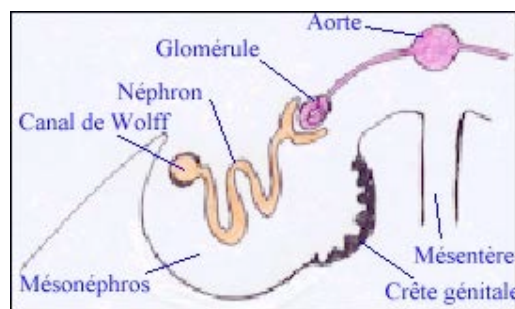
Différentiation sexuelle

- Au début de l'organogenèse, on observe la formation d'une crête génitale qui est colonisée par les cellules germinales. La crête génitale est issue (de même que les reins) du mésoderme intermédiaire, situé entre le mésoderme latéral et les somites.



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Relation avec le mésonephros

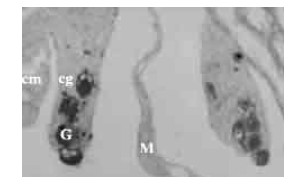
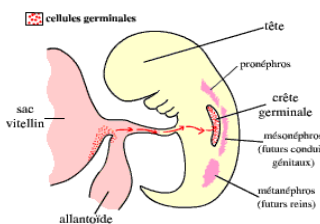


La gonade se présente comme un épaissement de la paroi du mésonephros.

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Différentiation sexuelle

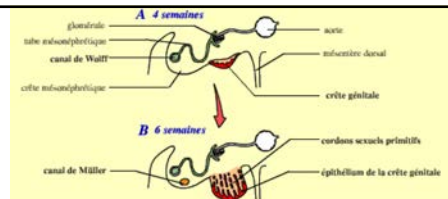
- Les cellules germinales migrent, chez tous les Vertébrés, pour aller coloniser la crête génitale. Chez les Mammifères, ces cellules sont originaire du mésoderme extra-embryonnaire. Elles accomplissent leur migration en passant par l'allantoïde.



M : mésentère dorsal. *G* : cellules germinales. *cg* : crête génitale. *cm* : blastème (crête) mésonephrétique

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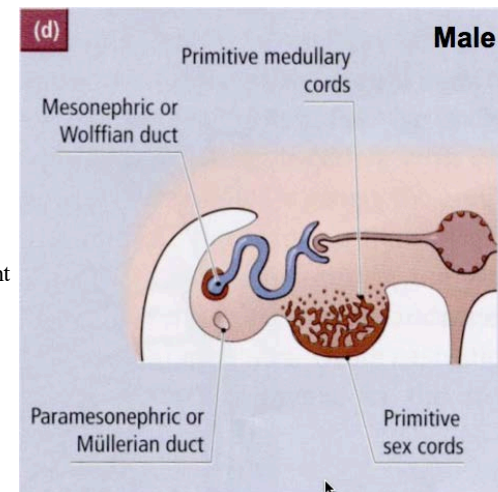
Différentiation sexuelle



- L' ébauche de la gonade apparaît, chez l'Homme, dans le mésoderme intermédiaire pendant la quatrième semaine de développement , et se développe d'abord de la même manière chez les deux sexes jusqu'à la septième semaine.
- L'épithélium de la crête génitale se développe dans le tissu conjonctif ; il se forme alors des cordons sexuels primitifs qui s'entourent de cellules germinales. Ces cordons sexuels vont proliférer jusqu'à la huitième semaine dans le tissu conjonctif.

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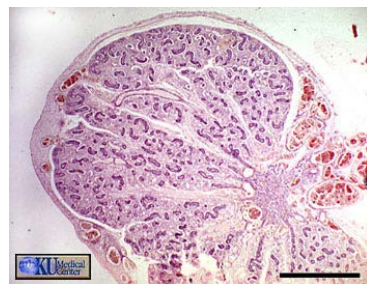
- Pour les foetus XY, on observe dans la crête génitale la formation de deux types de cordons : les cordons testiculaires et les cordons du rete testis qui se trouvent à l'extrémité des cordons testiculaires.



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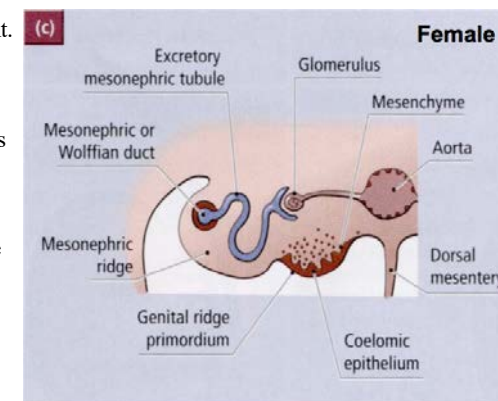
Testicule embryonnaire

Testicule humain (5 mois 1/2) : On peut voir l'albuginée (tissu entourant le testicule, dense et fibreuse), contenant des vaisseaux sanguins en développement, ainsi que les tubes séminifères en formation, convergeant vers la droite de la photographie. La barre mesure 1 mm.



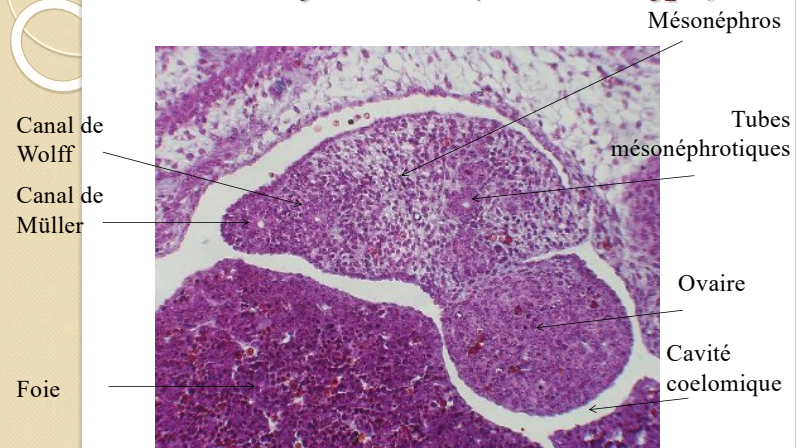
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- Chez les foetus de type femelle ou XX, les cordons sexuels primitifs dégénèrent. Néanmoins, l'épithélium de surface produit de nouveaux cordons qui ne pénètrent pas dans le tissu conjonctif mais qui restent en contact avec la surface corticale de la crête génitale



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Ovaire embryonnaire (souris 13 jpc)



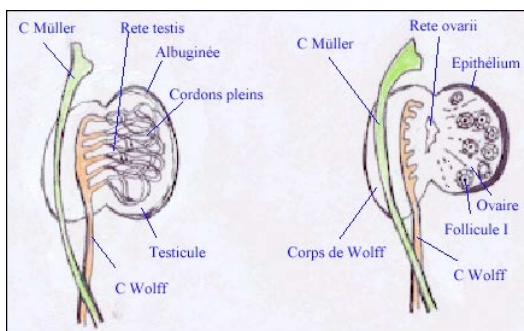
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Nombre de gonades

- À l'origine paire, elles peuvent fusionner (lamproies et myxines males et femelles) ou bien l'une peut dégénérer (raies) ou devenir abortif (ovaire droit des oiseaux sauf certains rapaces).

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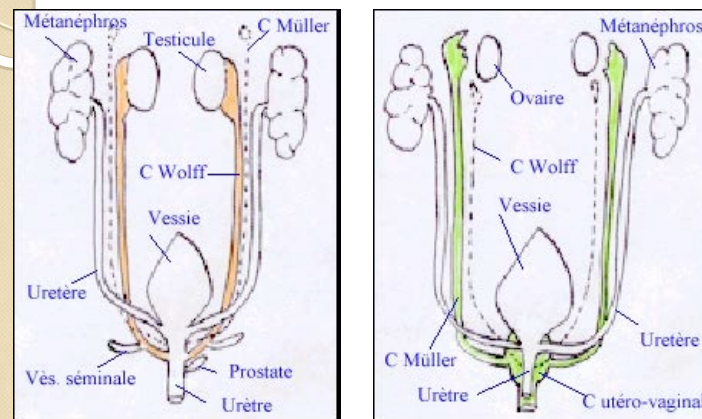
Organisation spatiale de la gonade et des canaux



Le canal de Müller s'édifie chez les deux sexes, mais il régresse chez les mâles.

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Adulte



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Position des gonades

- L'ovaire garde une position équivalente à sa position embryonnaire, intra-abdominale.
- Chez les non-mammaliens, le testicule reste intra-abdominal (position cryptorchide), de même que chez certains mammifères (éléphant, cétacés).
- Chez les autres, le testicule émigre à l'extérieur de la cavité abdominale, dans un diverticule coelomique (exorchidie, permanente chez l'homme, le cheval, les carnivores ou temporaire chez les rongeurs, les lémuriers).

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Vessie et cloaque

- À leur extrémité périphérique, les uretères débouchent soit directement dans le cloaque, soit dans une vessie elle-même en communication avec le cloaque par l'urètre.
- Sous le même nom de vessie, on désigne des structures d'origine très différentes.

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Les vessies

- Évagination cloacale (vessie cloacogène) ou, en partie, une dilatation du pédicule allantoïdien (vessie allantoïdienne des amniotes).
 - Dans ces deux cas, elle est essentiellement d'origine endodermique
- Elle peut aussi correspondre à une dilatation de l'extrémité périphérique des canaux excréteurs; elle est alors mésodermique.

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◦ Evolution de la détermination du sexe

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Quelques définitions

- Sexe primaire ou gonadique
- Sexe secondaire

- Détermination du sexe
- Différenciation du sexe
- Identification du phénotype sexuel

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Categories of sex determination

GSD: Genotypic sex determination
Sexual phenotype is influenced only by sex chromosomes

ESD: Environmental sex determination
Sexual phenotype is influenced by environmental conditions


TSD: Temperature-dependent sex determination
Sexual phenotype is influenced by temperature

TSD est un cas particulier d'ESD

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Sex determination in vertebrates

- First indirect identification of sex chromosomes by Morgan (1905) in *Drosophila* but the sex determination by itself is still thought to be largely influenced by environment even in mammals.
- The sex chromosomes were finally clearly identified in human in 1956.



♀ Femme		♂ Homme	
	X		X
	X		Y
1 2 3 4 5 6	23e paire: XX	1 2 3 4 5 6	23e paire: XY
7 8 9 10 11 12		7 8 9 10 11 12	
13 14 15 16 17 18 19 20 21 22		13 14 15 16 17 18 19 20 21 22	
22 paires d'autosomes et XX		22 paires d'autosomes et XY	



Ford, C. E. & Hamerton, J. L. 1956. The chromosomes of man. Nature, 178, 1020-1023.

47

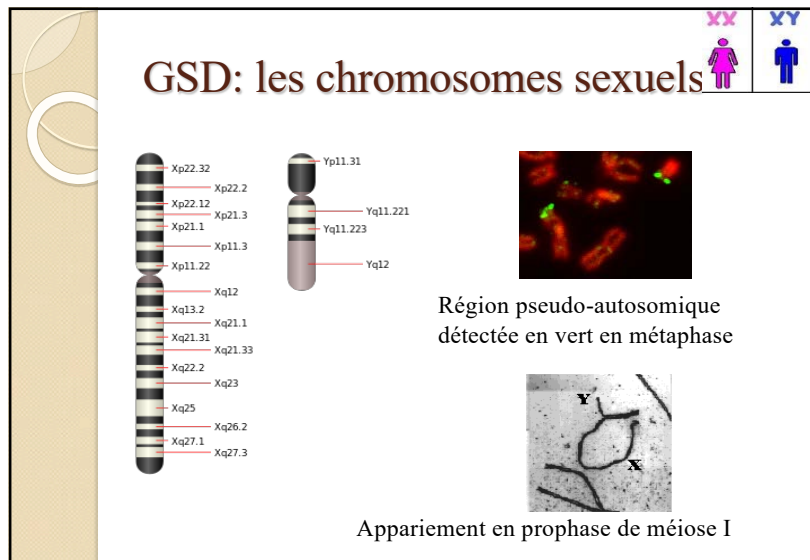
Croisements

	M	
F	X	Y
X	XX	XY
X	XX	XY
	F	M

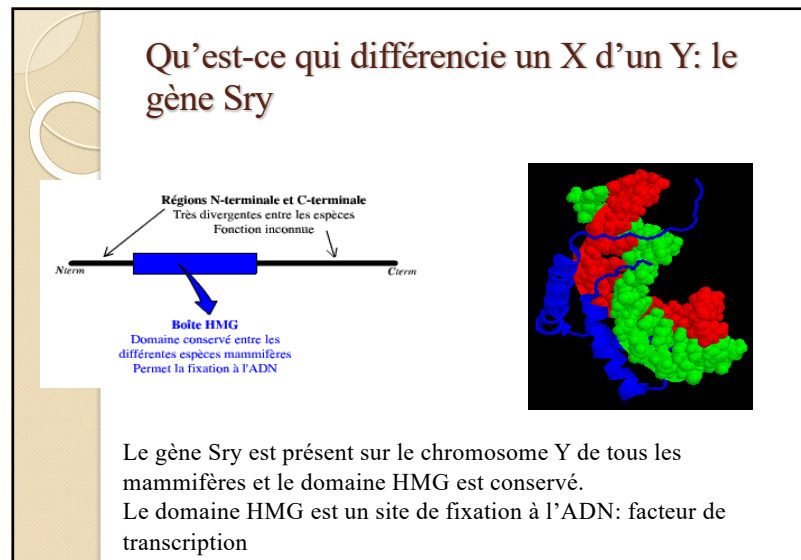
	M	
F	Z	Z
Z	ZZ	ZZ
Z	ZZ	ZZ
	W	F

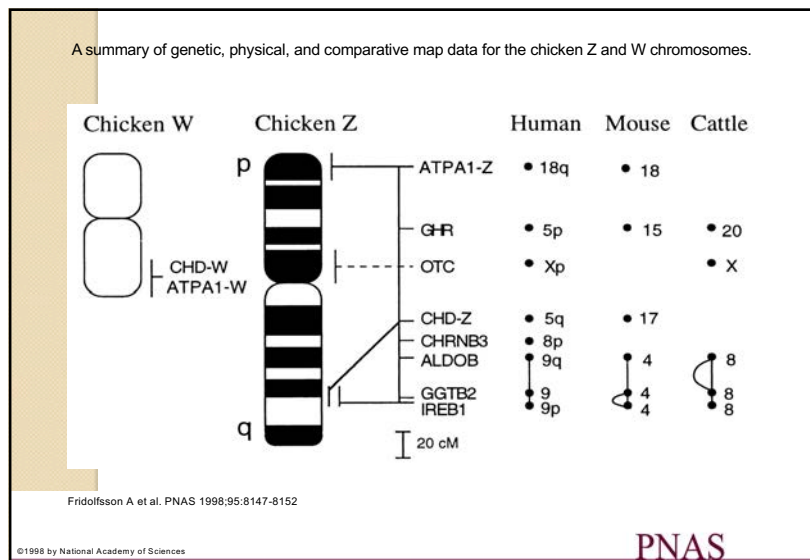
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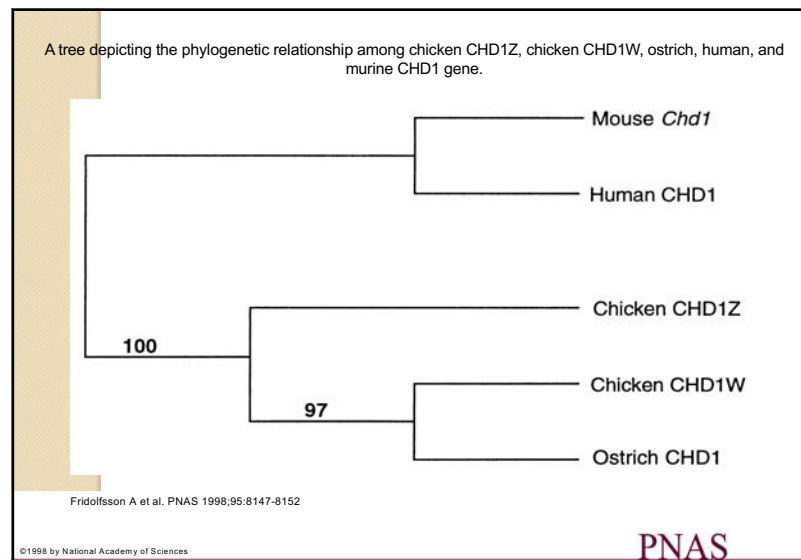
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Chromosomes sexuels

- XY ou ZW selon quel est le sexe hétérogamétique
- Les chromosomes X et Y ou Z et W d'une espèce descendent d'une paire d'autosomes mais il n'y a pas de relations entre XY et ZW: apparitions indépendantes

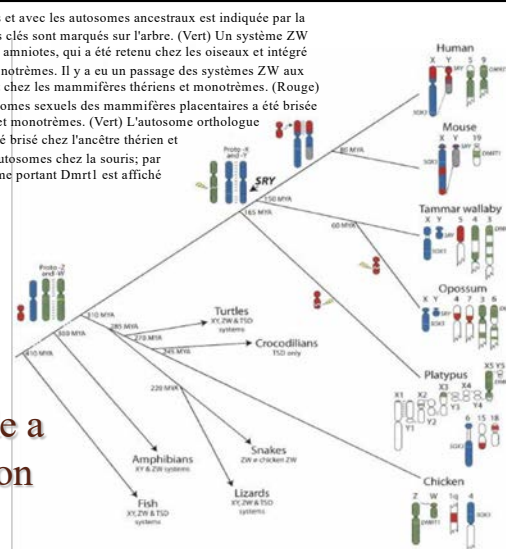
Susumu Ohno
February 1, 1928–
January 13, 2000



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L'homologie entre les génomes et avec les autosomes ancestraux est indiquée par la même couleur. Les événements clés sont marqués sur l'arbre. (Vert) Un système ZW semble être ancestral à tous les amniotes, qui a été retenu chez les oiseaux et intégré aux chromosomes chez des monotrèmes. Il y a eu un passage des systèmes ZW aux systèmes XY indépendamment chez les mammifères placentaires et monotrèmes. (Rouge) La région ajoutée aux chromosomes sexuels des mammifères placentaires a été brisée indépendamment en opossum et monotrèmes. (Vert) L'autosome orthologue au chromosome d'oiseau Z a été brisé chez l'ancêtre thérien et ensuite brouillé sur plusieurs autosomes chez la souris; par conséquent, seul le chromosome portant Dmrt1 est affiché dans cette espèce.

Evolution de la détermination du sexe



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Découverte de TSD chez les reptiles

- Charnier M, 1966. Action de la température sur la sex-ratio chez l'embryon d'*Agama agama* (Agamidae, Lacertilien). C. R. Soc. Biol. Paris 160: 620-622.



Madelaine Chamier, 1919-2002




Gabon, mai 2008


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TSD chez les chéloniens

- Pieau C, 1969. Sur une anomalie des conduits génitaux observée chez des embryons de Tortue grecque (*Testudo graeca* L.) traités par le benzoate d'oestradiol. C. R. Acad. Sci. (Paris) **268**: 364-367.
- Pieau C, 1970. Effets de l'oestradiol sur l'appareil génital de l'embryon de tortue mauresque (*Testudo graeca* L.). Arch. Anat. Microsc. Morph. Exp. **59**: 295-318.
- Pieau C, 1971. Sur la proportion sexuelle chez les embryons de deux Chéloniens (*Testudo graeca* L. et *Emys orbicularis* L.) issus d'oeufs incubés artificiellement. C. R. Acad. Sci. Paris **272(D)**: 3071-3074.
- Pieau C, 1972. Effets de la température sur le développement des glandes génitales chez les embryons de deux Chéloniens, *Emys orbicularis* L. et *Testudo graeca* L. C. R. Acad. Sci. Paris **274(D)**: 719-722.



Emys orbicularis





Testudo graeca

Claude Pieau

56

TSD chez les crocodiliens



- Ferguson MWJ, Joanen T, 1982. Temperature of egg incubation determines sex in *Alligator mississippiensis*. *Nature* 296: 850-853.

57

TSD chez le sphénodon

- Cree A, Thompson MB, Daugherty CH, 1995. Tuatura sex determination. *Nature* 375: 543.

58

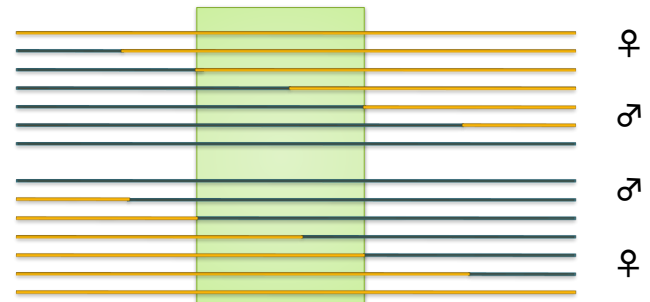
TSD chez les gnathostomes

	TSD	GSD
Actinopterygians	✓	✓
Amphibians	✓	✓
Amniotes		
• Sauropsida		
- Chelonians		
. Pleurodires	✓	✓
. Cryptodires	✓	✓
- Lepidosaurians		
. Rhyncocephales	✓	
. Squamates	✓	✓
- Archosaurians		
. Birds		✓
. Crocodilians	✓	✓
• Mammalians		✓

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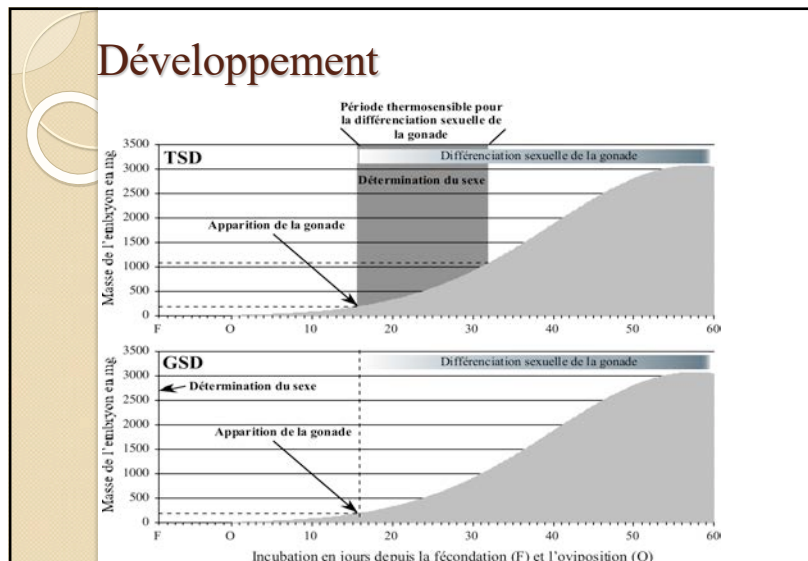
Quand la température agit-elle durant le développement ?

- Expériences de transfert d'œufs à différentes températures

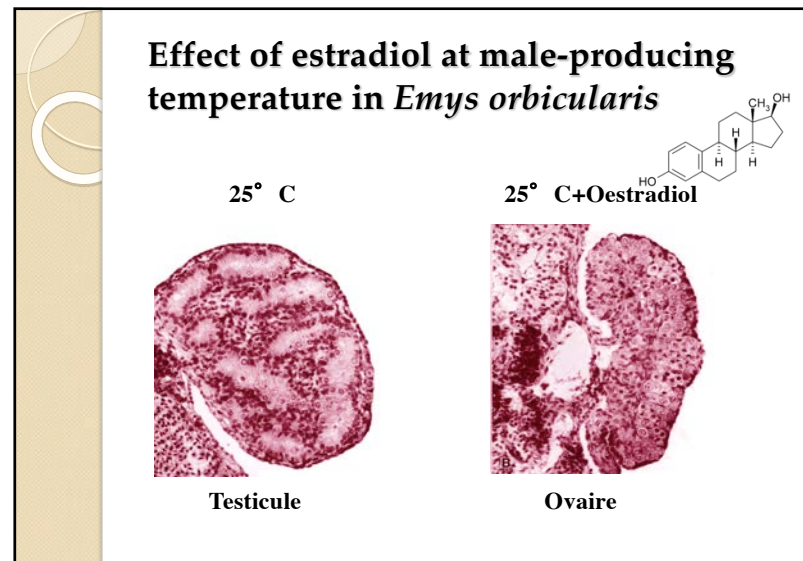


Picau, C. and Dorizzi, M. 1981. Determination of temperature sensitive stages for sexual differentiation of the gonads in embryos of the turtle, *Emys orbicularis*. - *J. Morph.* 170: 373-382.

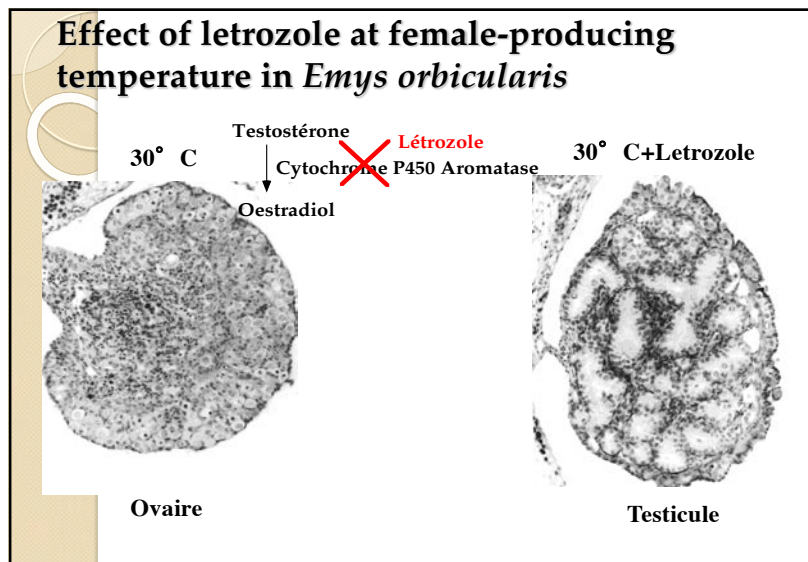
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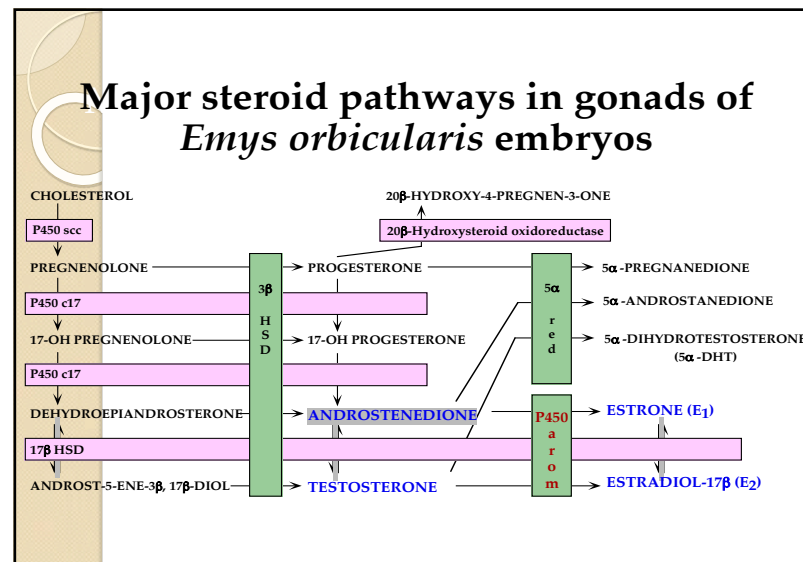
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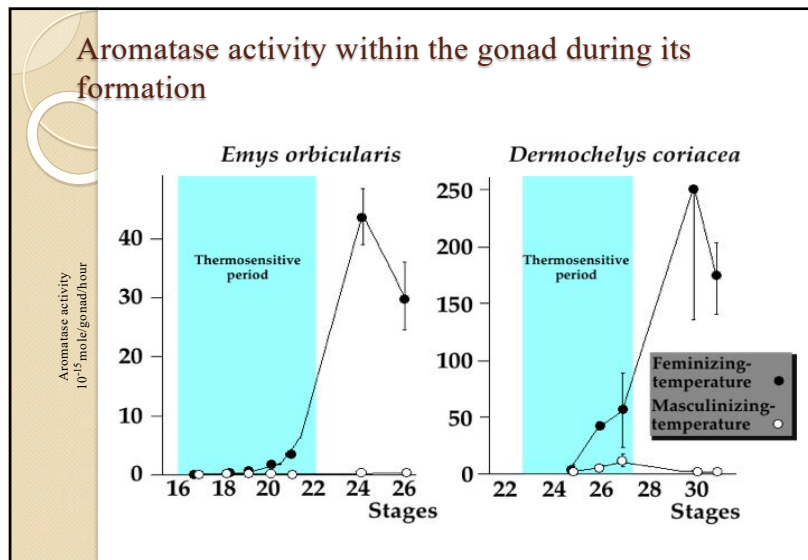
62



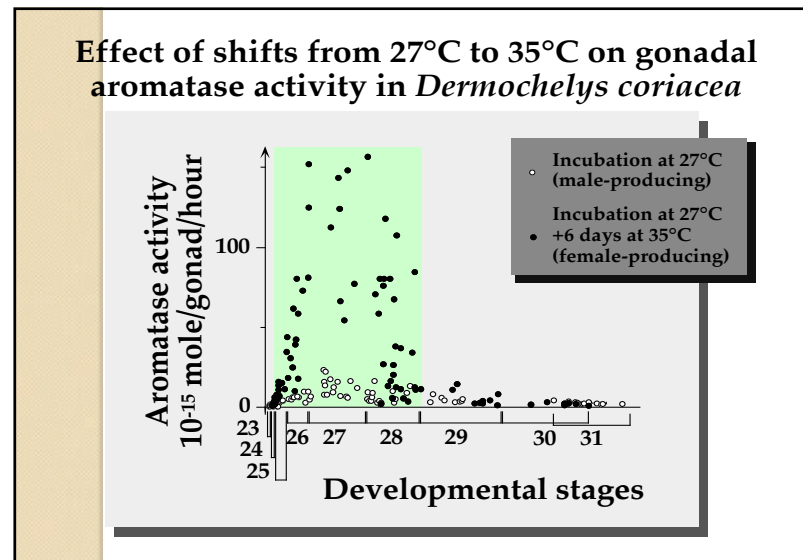
63



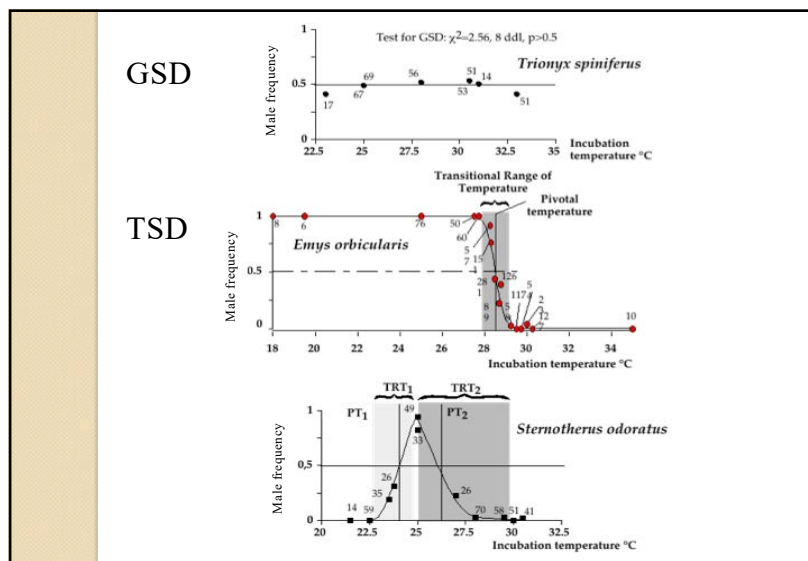
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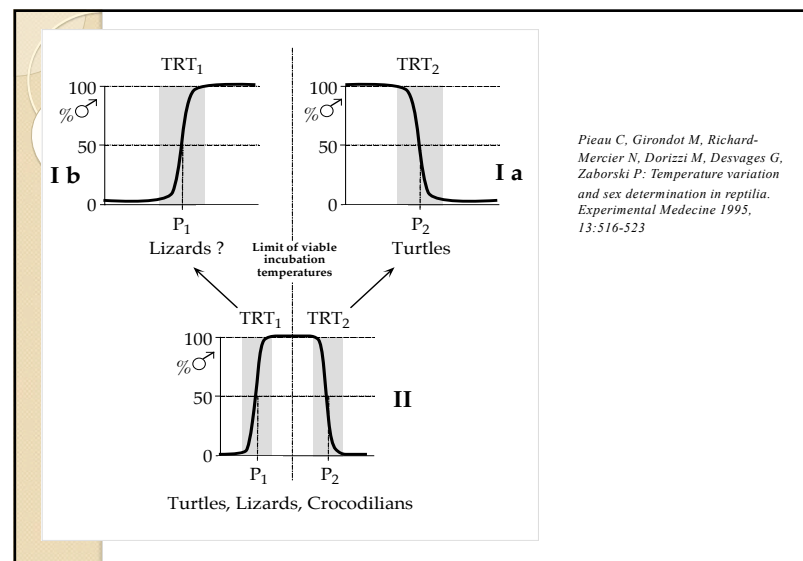
65



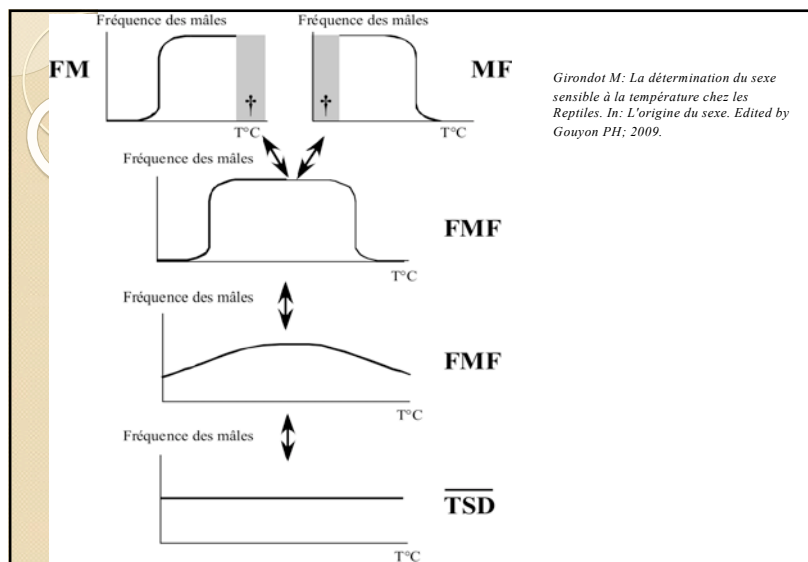
66



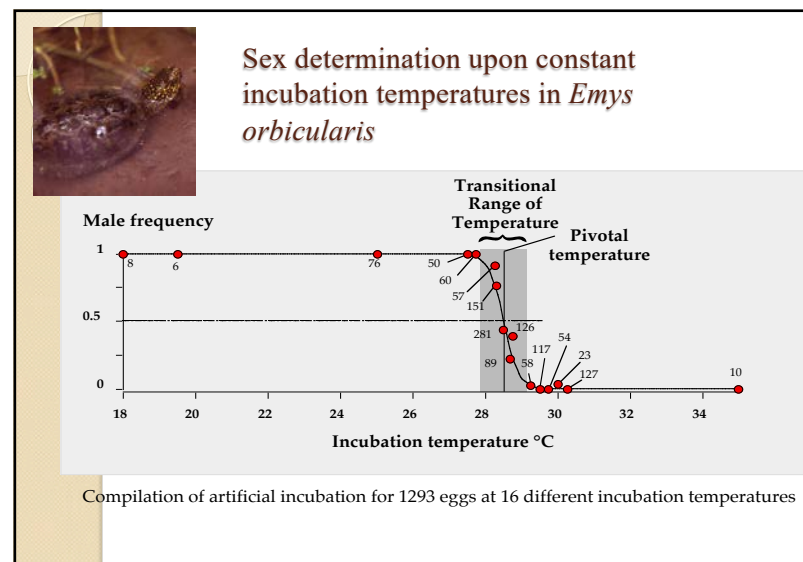
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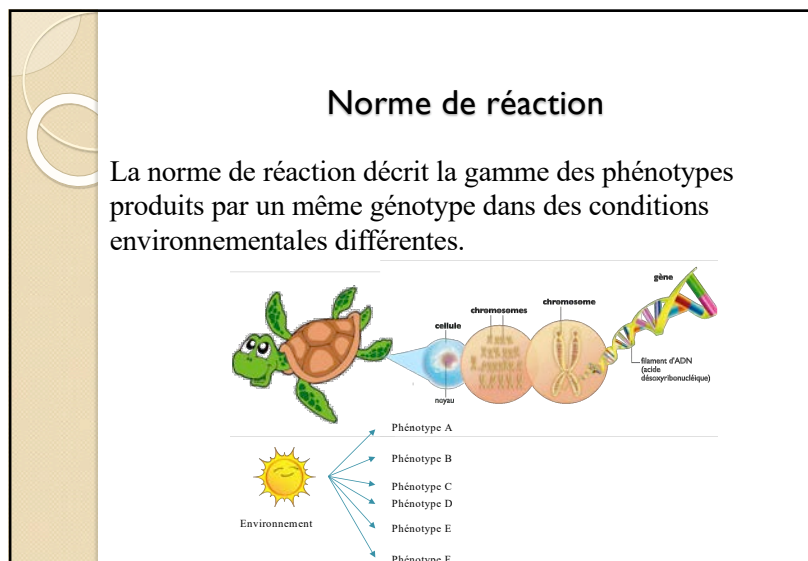
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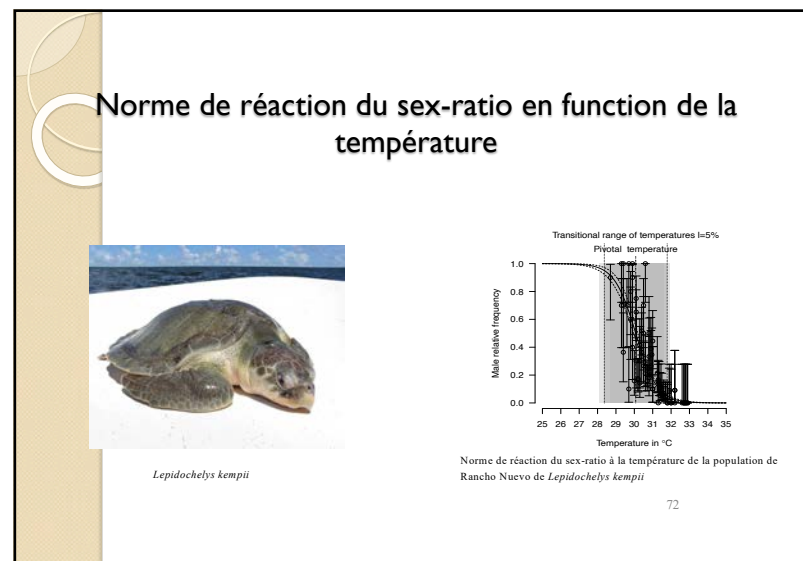
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71



72

Pourquoi étudier l'évolution de P et TRT ?

IPCC 2018

Observed monthly global mean surface temperature (1850-2014) and modeled responses to different anthropogenic emission and forcing pathways (2010-2100). The graph shows a clear upward trend in temperature over time, with various scenarios for future projections.

Transitional range of temperatures $I=5\%$

Male relative frequency vs Temperature in °C. The curve shows a sigmoidal transition from 1.0 to 0.0 between approximately 28°C and 32°C. A shaded region indicates the transitional range of temperatures where the frequency is between 0.5 and 0.95.

Solutions évolutives

- Adaptation du comportement maternel
- Adaptation de P
- Adaptation de TRT

73

73

Questions

- Existe-t-il un signal phylogénétique pour décrire l'évolution de P et TRT ?
 - Si oui, quand est-il visible sur une phylogénie ?
- Quelles variables environnementales peuvent constituer/ refléter des pressions de sélection pour P et TRT ?
 - La taille des femelles
 - La latitude du site de ponte

74

74

- Estimation de P et de TRT pour 43 groupes de tortues
- Calcul du signal phylogénétique par l'indice de Moran pour chacun des 50 000 réplicats
- Etude des corrélations entre l'évolution de P, TRT, la taille des femelles et la latitude

75

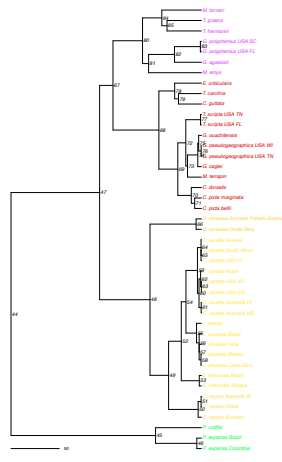
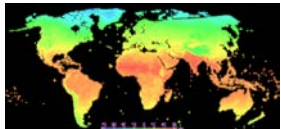

75

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76

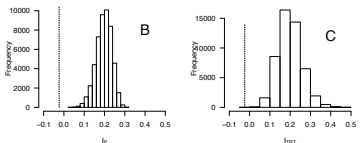
76

- Estimation de P et de TRT pour 43 groupes de tortues
- Calcul du signal phylogénétique par l'indice de Moran pour chacun des 50 000 réplicats
- **Etude des corrélations entre l'évolution de P, TRT, la taille des femelles et la latitude**

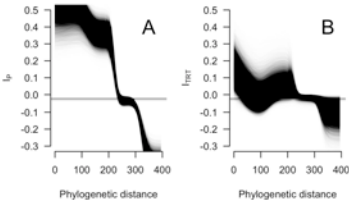




77

Signal phylogénétique



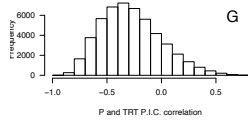
Existence d'un signal phylogénétique pour et TRT



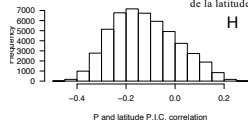
Détection d'un signal phylogénétique
Forte inertie évolutive pour P ;
Evolution rapide de TRT

78

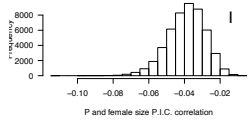
Contrastes phylogénétiquement indépendants – Evolution de P



Evolution de P indépendante de l'évolution de TRT



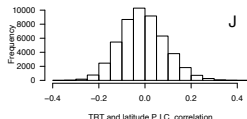
Evolution de P indépendante de la latitude de pont



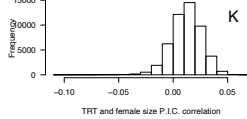
Non-indépendance de l'évolution de P et de la taille des femelles
P faible, grande taille des femelles

79

Contrastes phylogénétiquement indépendants – Evolution de TRT



Evolution de TRT indépendante de la latitude de pont ou de la taille des femelles



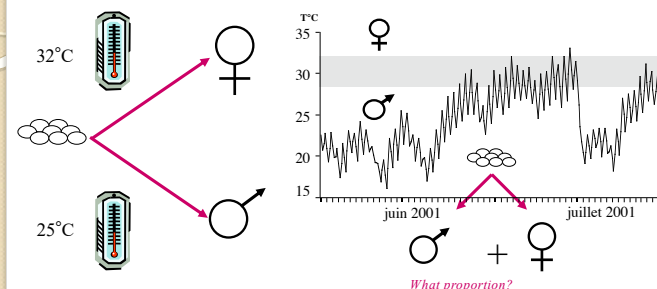
80

Conclusion

- P et TRT ont un signal phylogénétique
- P a une forte inertie évolutive, alors que TRT plus labile
 - L'évolution se ferait plus par un changement de la TRT qu'un changement de P
 - *Hulin et al. 2009* : les espèces qui ont une large TRT ont une plus forte proportion de nids produisant les deux sexes.

81

TSD: what we don't know...



Ecological puzzle

We don't know how to predict sex ratio in natural conditions

Evolutionary puzzle

We don't know why some species have TSD or GSD

82

Why we need a theory for the evolution of TSD?

- **Why Temperature-Dependent Sex determination occurs whereas genetic can do the work to provide Fisherian sex ratio very efficiently?**



- First indirect identification of sex chromosomes by Morgan (1905) in *Drosophila* but the sex determination by itself is still thought to be largely influenced by environment even in mammals.

- The sex chromosomes were finally clearly identified in human in 1956.

As a consequence, the idea of influence of environment in sex determination was replaced by a more "modern" genetic point of view.

83



Charnov -Bull hypothesis



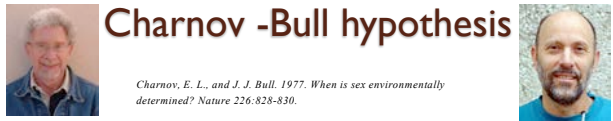
Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? *Nature* 226:828-830.

- Differential fitness for males and females according to the incubation temperature or temperature X sex effect on fitness
- Fitness of an individual is a measure of the relative capacity to transmit its own genes to the next generation (i.e. have descendants).
- Differential fitness is a consequence of selection process.
 - Selection can act between all individuals of the population: **this is the natural selection.**
 - Selection can act between males for access to females or between females for access to males: **this is the sexual selection.**

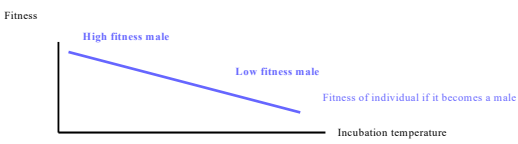
84

Charnov -Bull hypothesis

Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? Nature 226:828-830.



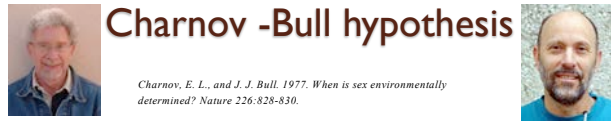
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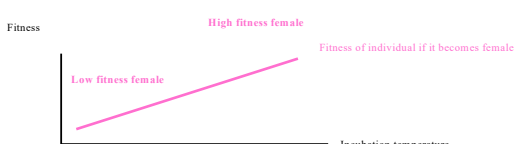
85

Charnov -Bull hypothesis

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86

Charnov -Bull hypothesis

Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? Nature 226:828-830.



- Differential fitness for males and females according to the incubation temperature
- or temperature X sex effect on fitness


Let 3 mutants being in competition:

- A Tendancy to be male at low T and female at high T
- B Tendancy to be female at low T and male at high T
- C Sex chromosomes, insensitive to incubation T

87

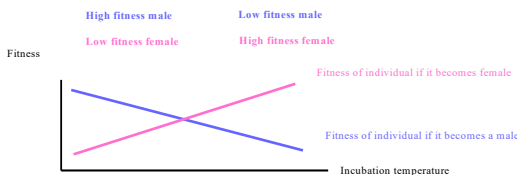
Charnov -Bull hypothesis

Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? Nature 226:828-830.



Mutants

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88

Charnov -Bull hypothesis

Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? Nature 226:828-830.

Mutants

- A Tendency to be male at low T and female at high T
- B Tendency to be female at low T and male at high T
- C Sex chromosomes, insensitive to incubation T

Mutants

Low temperature High temperature

Male Female Male Female

Fitness

High fitness male High fitness female

Low fitness female Low fitness male

Incubation temperature

89

Charnov -Bull hypothesis

Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? Nature 226:828-830.

Mutants

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- B Tendency to be female at low T and male at high T
- C Sex chromosomes, insensitive to incubation T

Mutants

Low temperature High temperature Low temperature High temperature

Male Male Female Female

The mutant **A** will invade the population and replace all other mutants. The population will have TSD MF. No other mutant can invade the population: the strategy TSD MF will be an **Evolutionary Stable Strategy, ESS.**

90

Charnov -Bull hypothesis

Charnov, E. L., and J. J. Bull. 1977. When is sex environmentally determined? Nature 226:828-830.

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91

Tentative of test... Janzen 1995

Test for survival difference between hatchling males and females

Male vs female differential survivorship

Temperatures

Male fitness Female fitness

Janzen, F. J. 1995. Experimental evidence for the evolutionary significance of temperature-dependent sex determination. *Evolution* 49:864-873.

92

Test for survival difference between hatchling males and females

- Wrong experimental design: Selection for TSD acts among males and among females, not between males and females.

Janzen, F. J. 1995. Experimental evidence for the evolutionary significance of temperature dependent sex determination. *Evolution* 49:864-873.

93

Test for survival difference between hatchling males and females

- Selection for TSD acts among males and among females, not between males and females.

Compilation of artificial incubation for 1293 eggs at 16 different incubation temperatures

94

The proposed solutions...

- Species with GSD to test for temperature X sex fitness hypothesis
- Estradiol-feminized females incubated at male producing temperatures vs. Females incubated at female-producing temperatures

95

GSD species to test for temperature effect on fitness

- Shine, R., M. J. Elphick, and P. S. Harlow. 1995. Sisters like it hot. *Nature* 378:451-452.

It is rather difficult to admit that this observation in a species with GSD proves that Charnov-Bull hypothesis for the evolution of TSD is valid... because the species has not acquired TSD !

96

Hormonal manipulation of sexual phenotype

- Freedberg, S., M. A. Ewert, and C. E. Nelson. 2001. Environmental effects on fitness and consequences for sex allocation in a reptile with environmental sex determination. *Evolutionary Ecology Research* 3:953-967.

97

Hormonal manipulation of sex ratio

Estradiol is naturally present in only some organs during embryogenesis. The hormonal manipulation of the entire embryo creates developmental anomalies.

Transverse sections through Wolffian duct (WD) and Müllerian duct (MD) of *Emys orbicularis* embryos at stage 23, incubated at male-producing temperature (25° C).

Girondot et al. 1998. Conserv. Biol.

98

Major problem with the Charnov-Bull hypothesis

- The temperature acts on sex determination during 20 days of the life of an individual which lasts around 19500 days.

99

THE SM hypothesis

Schwanz L.E., Cordero G.A., Charnov E.L., Janzen F.J. 2016 Sex-specific survival to maturity and the evolution of environmental sex determination. *Evolution* 70, 329-341. (doi:10.1111/evo.12856).

100

Life-history in the model

- No annual survival difference between males and females
 - The survival until sexual maturity at age A is reached is simply S^A ,
- However, being incubated at high or low temperature can make a strong difference in fitness,
 - Let S_c being is the annual survivorship for hatchlings incubated in c patch and S_h being the annual survivorship for hatchlings incubated in h patch.
- The age at maturity is reached at α_{males} and α_{females} respectively.
- Let the proportion of males in cold and hot patches being r_c and r_h for wild type and R_c and R_h for a mutant.
- What are the conditions for R to invade the population?

101

Conditions for TSD to evolve

- (1) age at maturity differs for males and females (e.g. $\alpha_{\text{males}} < \alpha_{\text{females}}$)
- (2) an annual survival advantage accrues during immaturity to individuals developing in one patch over the other (e.g. $\beta > 1$)
- When cold patches are rare, cold patches always produce males ($r_c = 1$), whereas hot patches overproduce females ($r_h < 1/2$).
- When hot patches are rare, hot patches always produce females ($r_h = 0$), whereas cold patches overproduce males ($r_c > 1/2$).
- At intermediate frequency of patches, cold patches produce all males and hot patches produce all females.
- **In all cases, cold patches produce more males than warm patches.**

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3 competing theories

Trivers-Willard, 1973
Charnov-Bull, 1977

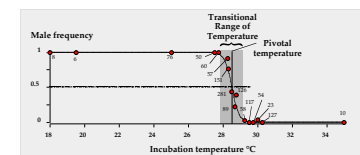
- TF (Temperature Fertility): TSD is favored when incubation temperature influences fertility differently for males and females
- TM (Temperature Maturity): TSD is favored when incubation temperature influences effective age at maturity differently for males and females
- SM (Survival Maturity): TSD is favored when annual juvenile survival rate depends on incubation temperature and the sexes mature at different ages

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The logic behind the TF and TM theories: ad-hoc hypothesis?

Males are observed at lowest temperature if lowest temperatures produce better males

Females are observed at highest temperature if highest temperatures produce better females



Compilation of artificial incubation for 1293 eggs at 16 different incubation temperatures

104

Are the SM hypotheses realistic?

- No juvenile survival difference between males and females
 - No sexual dimorphism is observed before the sexual maturity is reached thus no survival difference between males and females is expected,
- However, being incubated at high or low temperature can make a strong difference in fitness,
 - It is known that too low incubation temperature or too high are lethal.
- The age at maturity is reached at α_{males} and α_{females} years respectively.

105

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 - In turtles, most recent dataset shows that males mature earlier than females, between 0 to 8 years ($\alpha_{\text{males}} < \alpha_{\text{females}}$).
 - The age difference is one year greater for species with TSD than for species with GSD (but the difference is not significant)

106

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107

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108

What is still unknown?

- What are the expected primary, secondary and operational sex ratios at the ESS?
- How strict must be the hypotheses?
 - How the model works for continuous habitat, not patchy?

109

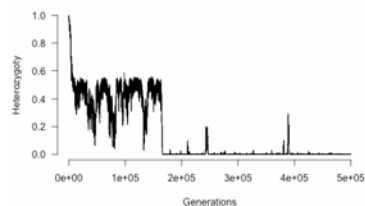
Individual Genetically-explicit Based Model

- $P=0.5$ Proportion of c and h habitat
- $S=0.9$ Annual survival after sexual maturity
- $S_c=0.9$ Annual survival before sexual maturity if incubated in c habitat
- $\beta=1.05$ $S_c \cdot \beta = 0.945 = S_h$ is the annual survival before sexual maturity if incubated in h habitat
- $\alpha_{\text{male}}=8$ Age at maturity for males, 8 years
- $A_{\text{female}}=15$ Age at maturity for females, 15 years
- $N_{\text{ind}}=1,000$ Constant population size (large to minimize drift)
- $N_{\text{years}}=500,000$ Number of years (large to let selection to operate)
- r is coded in one locus with 2 values for each allele: r_c and r_h
 - They represent the proportion of males in c habitat and proportion of males in h habitat. Additive effect for maternal and paternal allele. Mutation rate of 10^{-5} per year.
 - GSD is therefore coded as $r_c=0.5$ and $r_h=0.5$
 - Initial heterozygosity is 1

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Change in heterozygosity

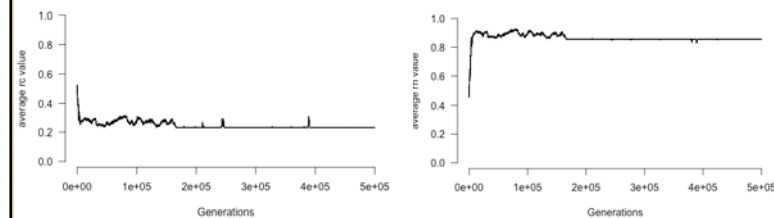
- An allele is selected and it resists to the introduction of others: ESS



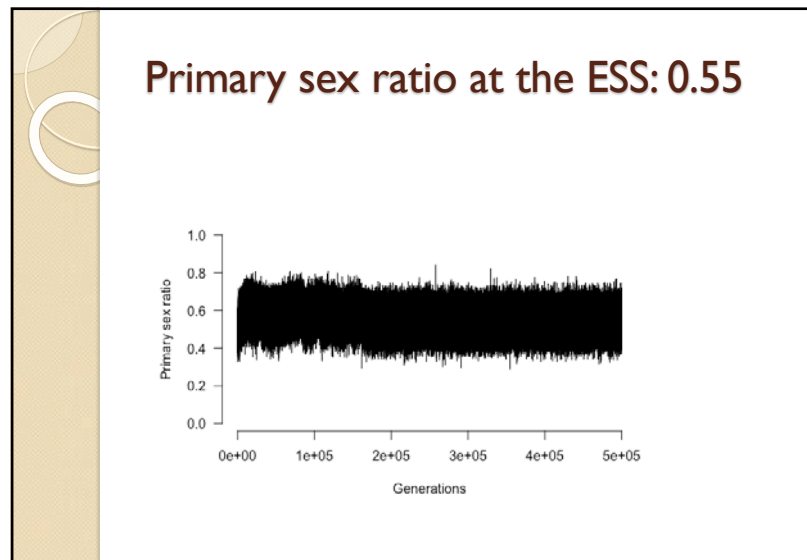
111

Selected allele: $r_h=0.85$ and $r_c=0.23$

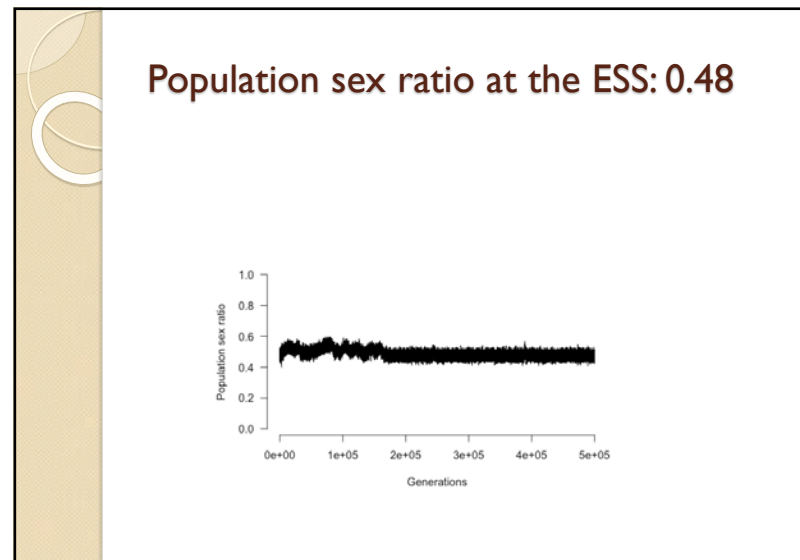
- More females in c habitat and more males in h habitat



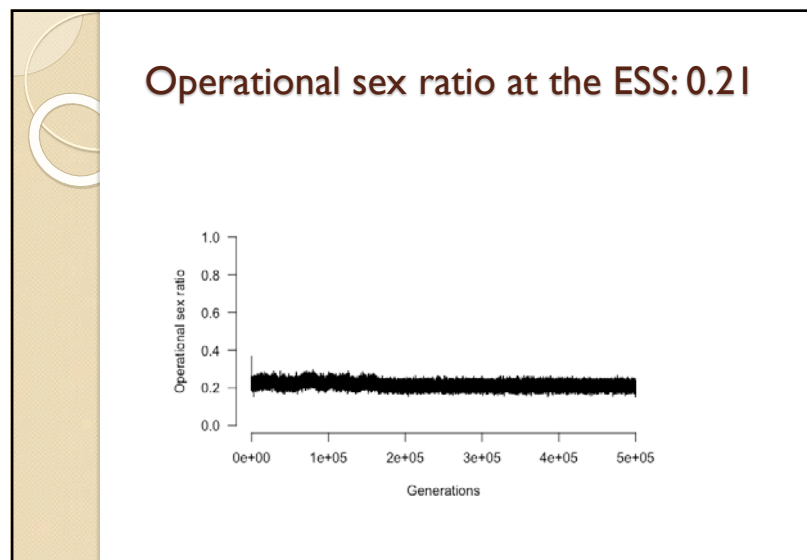
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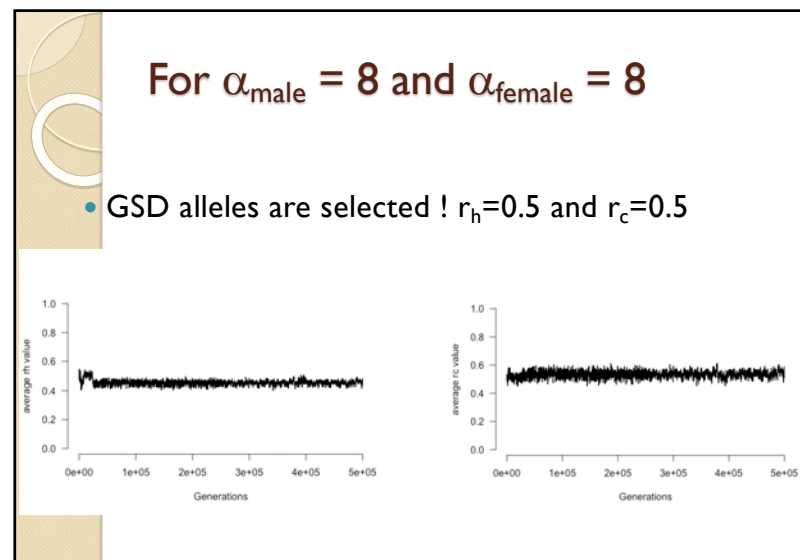
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114



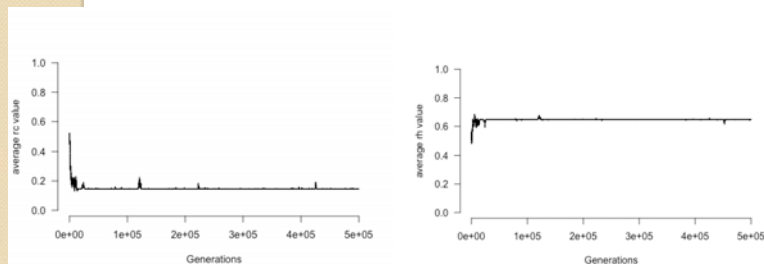
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For $\alpha_{\text{male}} = 8$ and $\alpha_{\text{female}} = 12$, with $P=0.2$

- TSD alleles are again selected, $r_h=0.65$, $r_c=0.15$



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Synthesis

	P=0.5		P=0.5		P=0.2
P=0.2	P=0.5		P=0.5		P=0.2
	$\alpha_m=8, \alpha_f=12$	$\alpha_m=8, \alpha_f=15$	$\alpha_m=8, \alpha_f=12$	$\alpha_m=8, \alpha_f=15$	$\alpha_m=8, \alpha_f=8$
Primary sex ratio	0.55	0.55	0.55	0.61	0.50
Population sex ratio	0.50	0.48	0.52	0.56	0.50
Operational sex ratio	0.22	0.21	0.22	0.24	0.50
r_h	0.88	0.85	0.65	0.75	0.50
r_c	0.23	0.23	0.15	0.04	0.50
	TSD				GSD

118

Conclusions

- The proposed model by Lisa Schwantz seems to be very pertinent to explain the occurrence of TSD in reptiles;
- For some species, after an initial selection, the TSD character could remain present as it is nearly neutral for long-lived species (not tested)

119

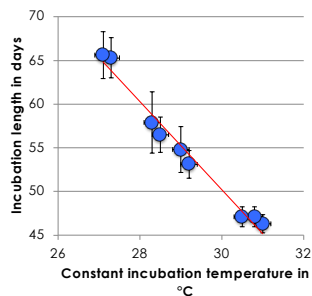
What is the best way to estimate sex ratio for natural nests?

120

Incubation length proxy...

- Rate of development in reptiles is dependent on integrative cumulated effect of temperature during development.

Caretta caretta incubation length
Mrosovsky et al. 2002



121

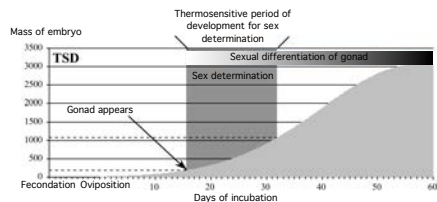
Incubation length proxy...

- Rate of development in reptiles is dependent on integrative cumulated effect of temperature during development.
- For species with TSD, sex is also dependent on integrative cumulated effect of temperature during thermosensitive period of development for sex determination.
- Then, it has been proposed to use the incubation length as an index of sex ratio.

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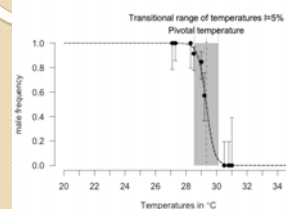
But...

- However, the development is dependent on temperature during the entire incubation whereas sex is dependent on temperature only during the so-called TSP, at the middle-third of incubation.

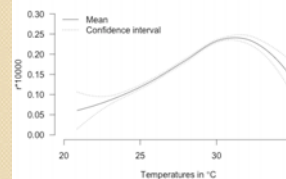


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More problematic...

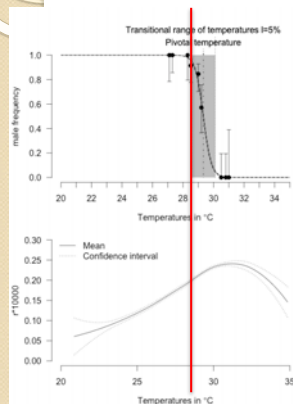


- TSD reaction norm and embryo growth reaction norm are not parallel



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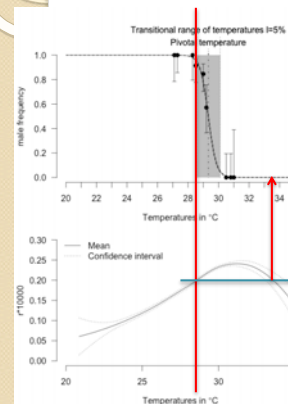
More problematic...



- TSD reaction norm and embryo growth reaction norm are not parallel

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More problematic...



- TSD reaction norm and embryo growth reaction norm are not parallel
- For a same incubation length, 100% males or 100% females can be

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But...

- **The correlation between sex ratio and incubation length is very good, so why it is not possible to use it?**
- Any biological activity is sensitive to temperature based on Arrhenius Principle:
 - Arrhenius' equation gives the dependence of the rate constant k of a chemical reaction on the absolute temperature T (in kelvins), where A is the prefactor, E_a is the activation energy, and R is the universal gas constant:

$$k = Ae^{-E_a/(RT)}$$

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The world of k s

- Each reaction has its own k value but as all these effects are linked by a single constant, T . As a direct consequence they seem correlated just because same T is used in all.
- However, using this correlation to predict sex ratio is very dangerous and can give completely wrong result.

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Constant-temperature equivalent weighted by embryo growth rate

- Using an embryonic growth rate model, we can estimate when is the TSP;
- But how to estimate sex ratio based on temperatures within TSP?
- Based on observations, Pieau (1974) has proposed that the longer the embryo develops at one temperature, the more this temperature will influence the sex
 - Rate of development being dependent on temperature, we must weight the contribution of each temperature during TSP by its impact for growth of embryo ...

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Constant-temperature equivalent

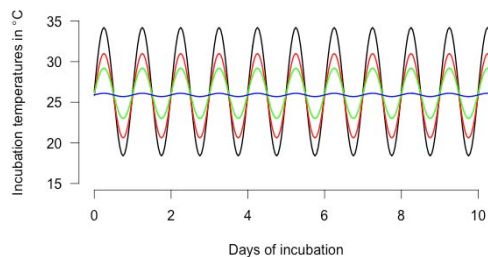
- Georges et al. (1989, 1994) have demonstrated this idea in an experiment with constant mean temperature but varying amplitude:
 - The highest the amplitude of temperatures, the more feminized are the embryos: in a cyclic pattern, higher temperatures have more influence than lower ones because embryo develop faster at these temperatures

Georges A. 1989. Female turtles from hot nests: Is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* 81:323-328.
 Georges A., Limpus C.J., Stoutjesdijk R. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *Journal of Experimental Zoology* 270:432-444.

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Constant-temperature equivalent

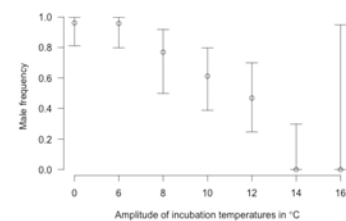
- Georges et al. (1989, 1994) have demonstrated this hypothesis in an experiment using incubation at constant mean temperature but varying amplitude.



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Constant-temperature equivalent

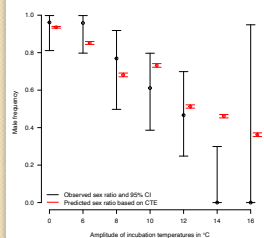
- The highest the amplitude of temperatures, the more feminized are the embryos: in a cyclic pattern, higher temperatures have more influence than lower ones because embryo develop faster at these temperatures



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CTE and sex ratio

- CTE were estimated taking into account only the plasticity on embryonic growth rate



- The conclusion is clear, it is not sufficient
- It confirms that embryonic development thermal reaction norm is not a good proxy of thermal reaction norm of sex ratio.

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The end of incubation length

- Then incubation length should not be used as a proxy of sex ratio for at least two reasons:
 - The thermal reaction norm of embryo growth is different from the thermal reaction norm of sex ratio even if they are correlated
 - The incubation length is dependent on temperature also out of the TSP period

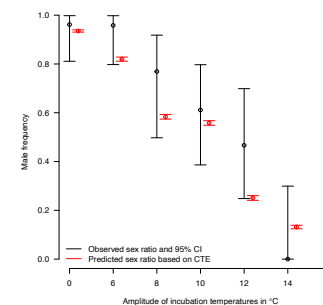
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What can we do?

- We must introduce another thermal reaction norm: **The thermal reaction norm for sexualisation**
- This thermal reaction norm relates the level of sexualisation of the gonad with the temperature. It is different from thermal reaction norm of sex ratio because thermal reaction norm for sex ratio is the same at 30 and 35 ° C whereas 35 ° C is a more feminizing temperature than 30 ° C and then the thermal reaction norm for sexualisation is higher.

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With this new thermal reaction norm



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