Inbreeding

Gavin Ramsay

Apis mellifera



Homo sapiens – Pedro II of Brazil



Aims

- Show that populations carry hidden versions of genes (=alleles)
- In humans, show that inbreeding ‘exposes’ these copies
- In bees, similar issues can be expected
- How mixing in populations keeps these issues at bay
- Bee biology has special features that also interact with inbreeding
- All this means we need to be careful when breeding bees

Genes, alleles, dominance, …..

Let there be a gene called C. It will sit at the C locus on a chromosome and it may have variants called alleles. Single individuals may be:

CC – homozygous for the dominant form of the gene

cc – homozygous for the recessive form of the gene (the individual ‘expresses’ or shows the c type)

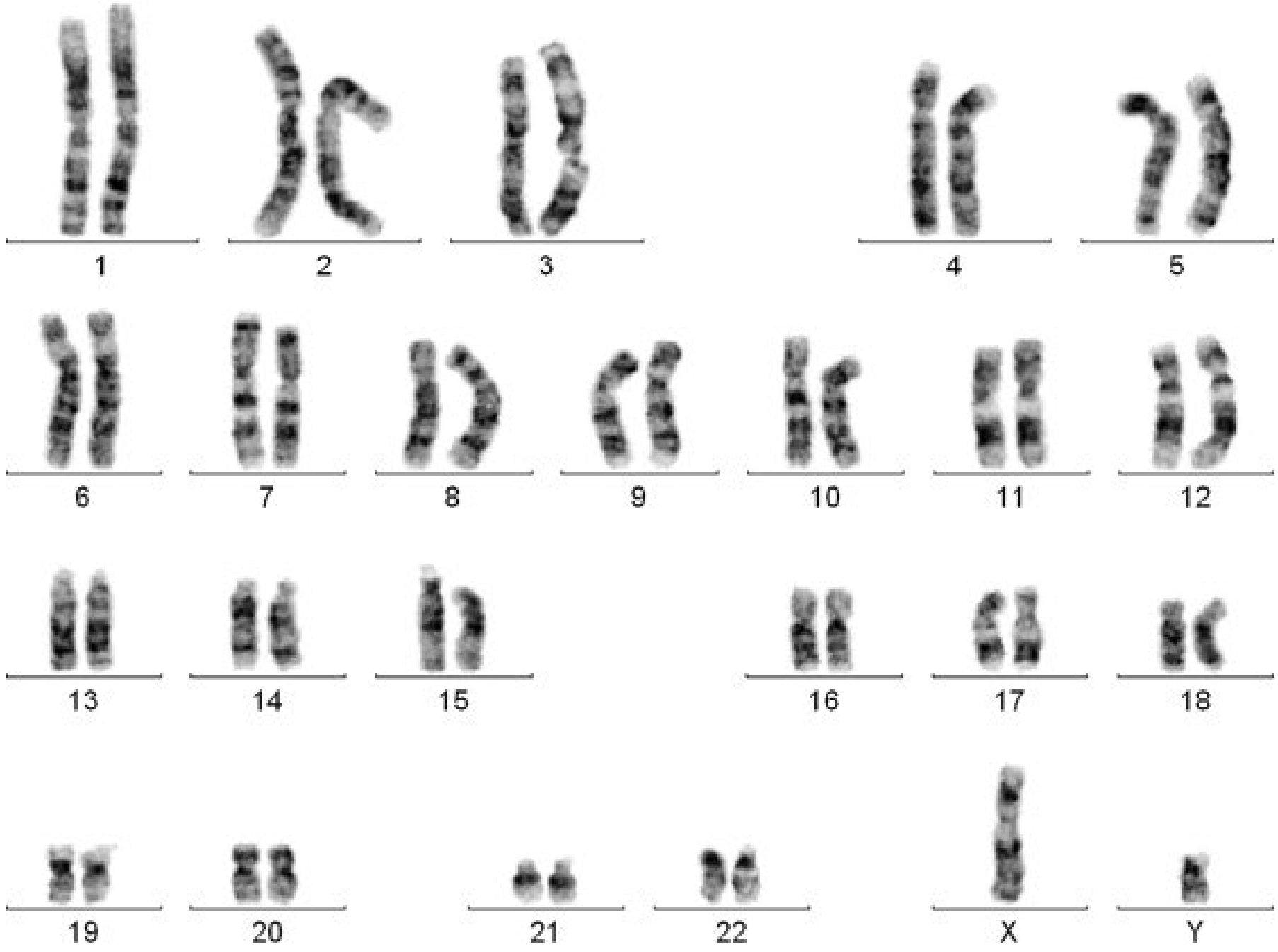
Cc – heterozygous for C and c (the effect of c is masked by the expression of the C allele)

Other dominant alleles (C_1, C_2, C_3, etc) and other recessive alleles may exist.

Alleles are often not fully dominant or recessive.

Genomes accumulate variants and faults

- Mutations accumulate in humans at about 100 per generation
- Most of these are not in coding sequence and have no effect
- Roughly one of these is ‘deleterious’ ie the gene fails to make its protein or makes a protein that will not function normally
- As deleterious mutations accumulate, individuals become less fit and more likely to succumb to disease – metabolic or infectious
  - Even when essentially recessive, there can be a deleterious effect in heterozygous individuals (ie both versions of the gene are present)


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X
Y

Data from the 1000 (Human) Genomes Project

Each human carries a range of deleterious mutations

- ‘missense’ mutations, changes in the genetic code
  - 281 – 515 per individual (12-22 per chromosome on average)*
  - of these 40-85 were homozygous, might be deleterious
- Disease-causing mutations
  - 40-110 per individual
  - 3-24 of these were in the homozygous state
- Very damaging mutations
  - 0-8 per individual
  - 0-1 of these are homozygous

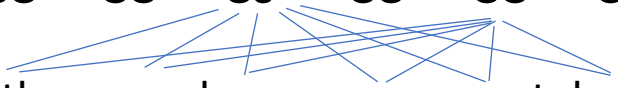
*other research suggests 1,000

Xue at al. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3516590/
Sung Chun, Justin C. Fay.
https://doi.org/10.1371/journal.pgen.1002240

Inbreeding at the genetic level

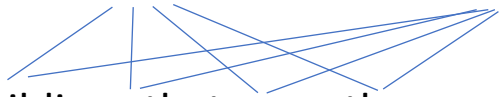
A gene, C, for some trait. Let's say a survivable response to covid. The recessive allele c is risky and rare

CC CC CC Cc CC CC CC CC CC CC CC CC CC CC CC



If the fourth person becomes a parent, half their offspring will carry the recessive allele

Cc CC Cc CC CC Cc



If two of these siblings that carry the recessive allele mate, one quarter of their offspring will carry two copies of the recessive allele ie will be homozygous for that 'bad' allele (and be at risk of bad things during covid).

CC Cc Cc cc

With each generation of inbreeding, this type increases and the heterozygous form Cc decreases

Inbreeding like this does two things:

1. It can 'fix' certain alleles in the population, by chance, especially if there was a bottleneck
2. It can expose recessive alleles that are normally obscured

Inbreeding

- Mating and producing offspring with related individuals
- Causes rare, recessive (and often ineffective) gene variants to be exposed
- Can also ‘fix’ traits in populations rather than permitting variability to persist (especially if there is a bottleneck in the population)
- Effects are often deleterious for the individual
- Also means that the population has less ability to adapt

Inbreeding

- Exposes deleterious alleles
- Allows rapid selection
- Reduces available diversity
- Fixes traits in a population
- Causes inbreeding depression
- New combinations of genes become tricky

Outbreeding

- Masks deleterious alleles
- Slows selection
- Maintains underlying diversity
- Inability to fix traits
- Gives hybrid vigour
- New combinations of genes remain a possibility

Inbreeding in humans

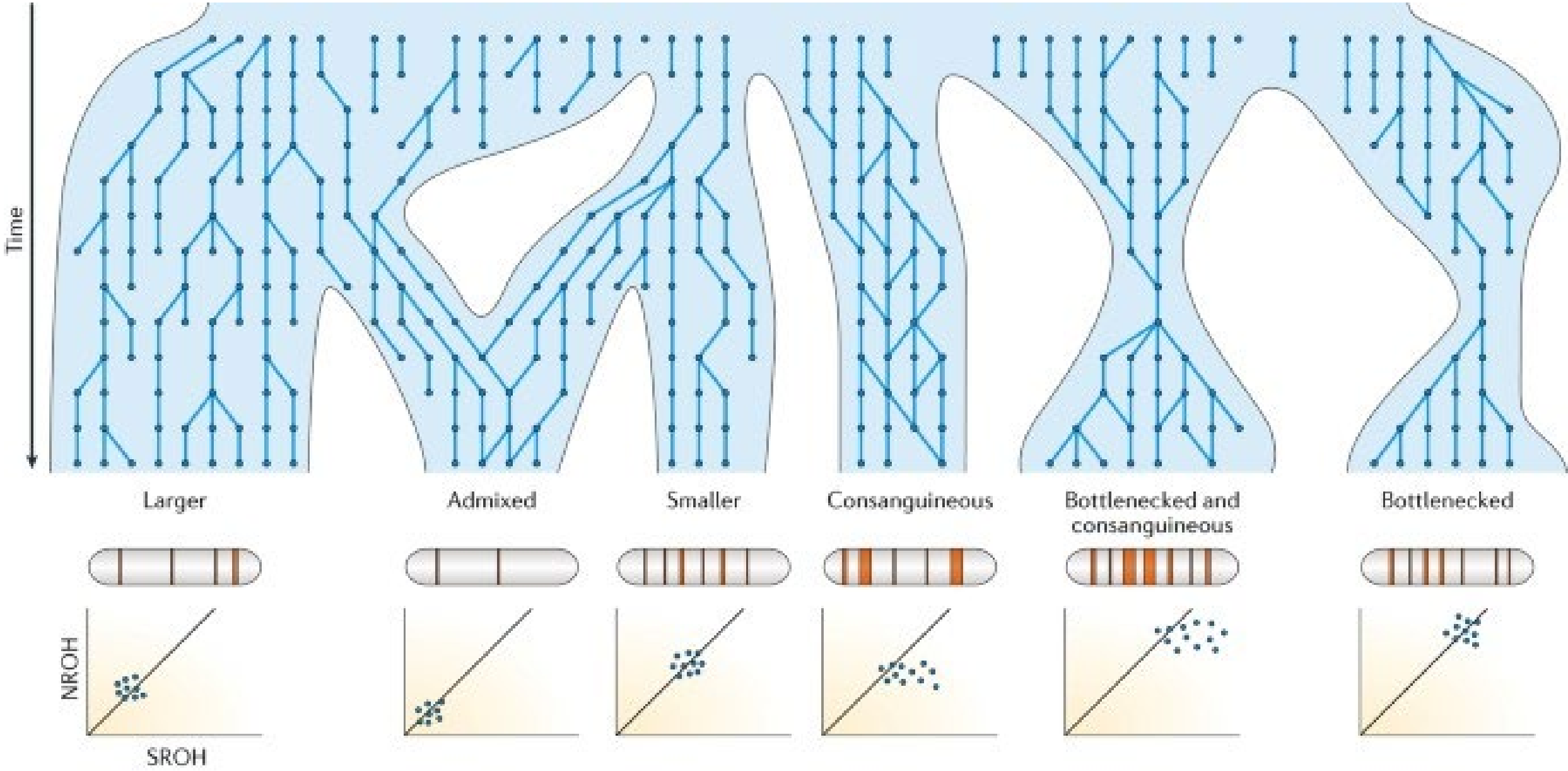
- When two related parents create offspring
- In human societies, cultural and social barriers are often in place
- Subtle aspects of mate selection can act to increase inbreeding
- Royal dynasties often indulge in inbreeding
  - keeping the riff-raff out  and maintaining family resources
  - Individuals are privileged, can be entitled and willing to break taboos
  - Creating a defensive ring in court of reliable (related) friends
- Other opportunities for inbreeding
  - Religious sects
  - Island communities
  - Isolated communities

What traits show inbreeding effects in humans?

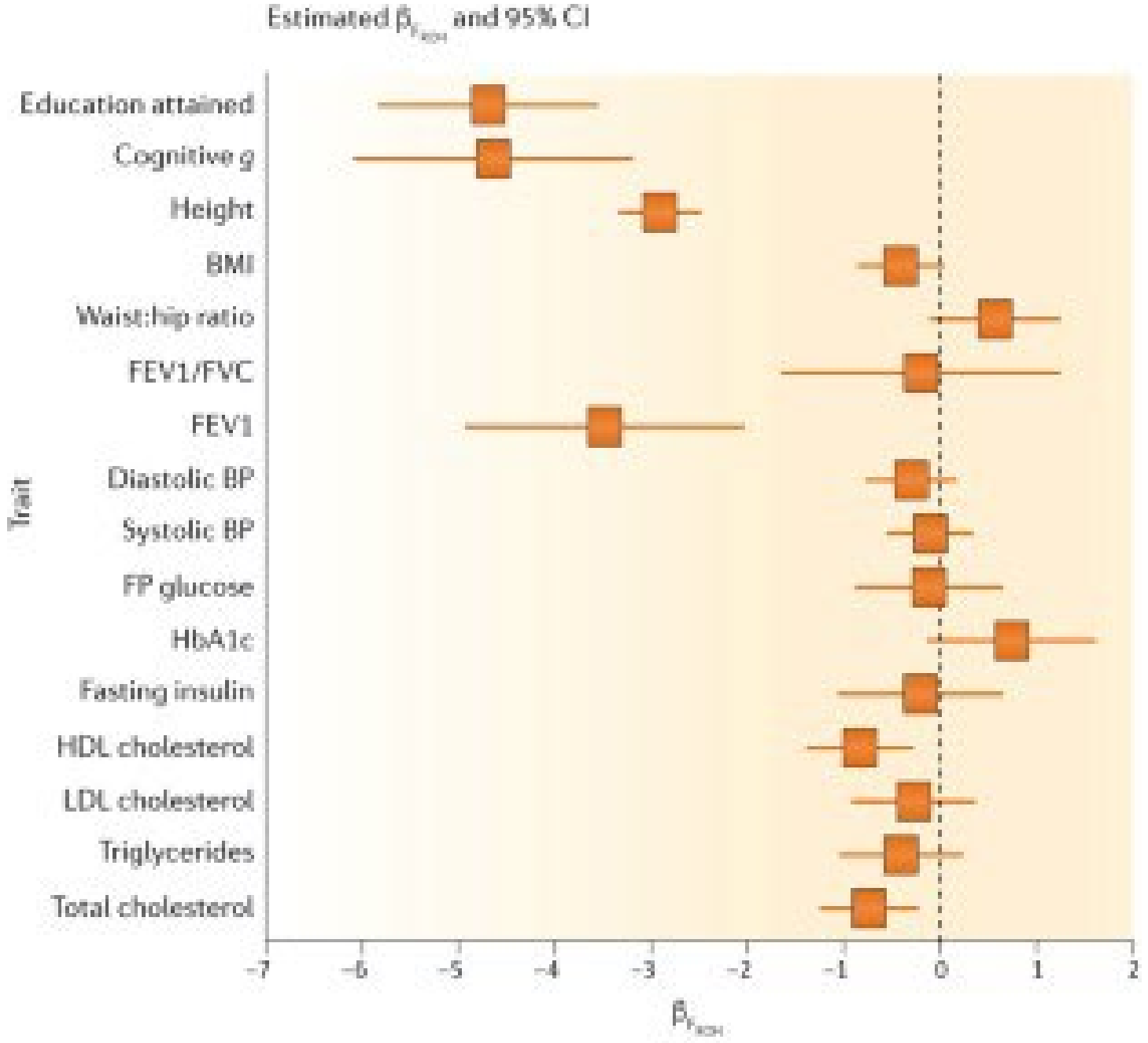
- *anything* can be affected by inbreeding, as all genes are susceptible
- Inbreeding can reduce vigour, height, intelligence, disease resistance, lung function, change morphological features, cause metabolic disease, reduce fertility, longevity
- Those traits that require a suite of genes to work together are most susceptible to inbreeding

Genome sequencing is permitting better resolution

- ‘Runs Of Homozygosity’ in chromosomes are now the leading way to assess how inbred a stock is
- How much of the genome is identical on the maternal and paternal chromosomes



Ceballos et al. 2018. https://www.nature.com/articles/nrg.2017.109



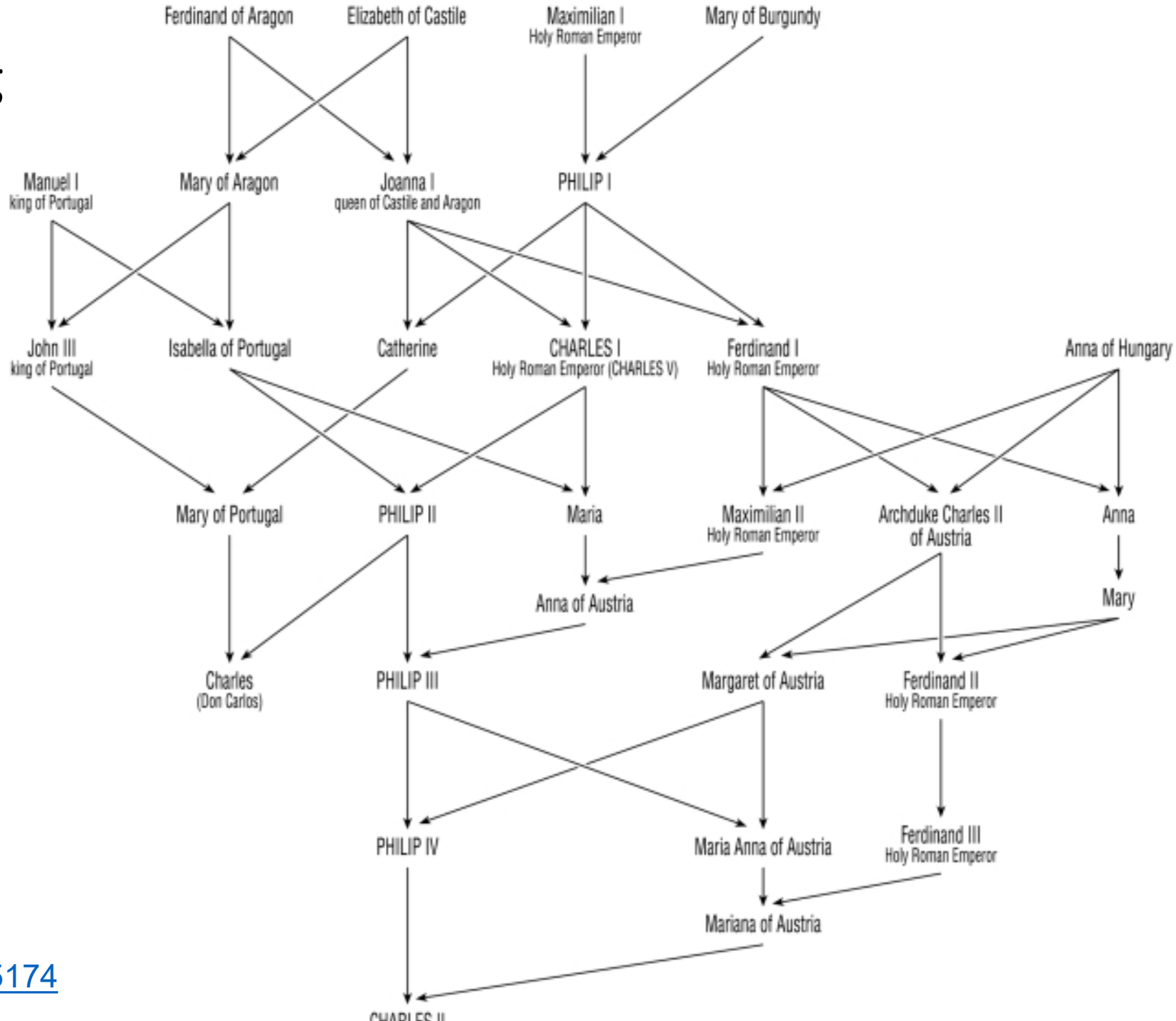
Ceballos et al. 2018. https://www.nature.com/articles/nrg.2017.109

The Habsburgs

- The Habsburgs comprised a dynasty of 6,000 individuals over 20 generations
- They were Royals in Austria, Belgium/Low Countries, Spain, Portugal, Hungary, Holy Roman Empire, Mexico, Brazil ….
- The family portraits often show the Habsburg's Chin – a protruding chin, an enlarged lower lip and a long, strong nose
- As with some other dynasties, inbreeding was common
- Inbreeding (as determined from pedigrees), facial deformities, other health issues, infertility and early death all reached a peak in the Spanish Habsburgs up to 1700 when this Royal line died out.
- Other non-Royal but Habsburgs of classic appearance died out in the 1700s with a few persisting into the 1800s.



The Spanish Habsburg Dynasty 1516-1700



Alvarez et al.

PLoS One. 2009; 4(4): e5174.

Published online 2009 Apr 15. doi: 10.1371/journal.pone.0005174

The Spanish Habsburg Dynasty 1516-1700



Charles II:
- his grandmother and his aunt were the same person
- his ancestors comprised a closed population
- they had indulged in inbreeding for many generations

Alvarez et al.

PLoS One. 2009; 4(4): e5174.

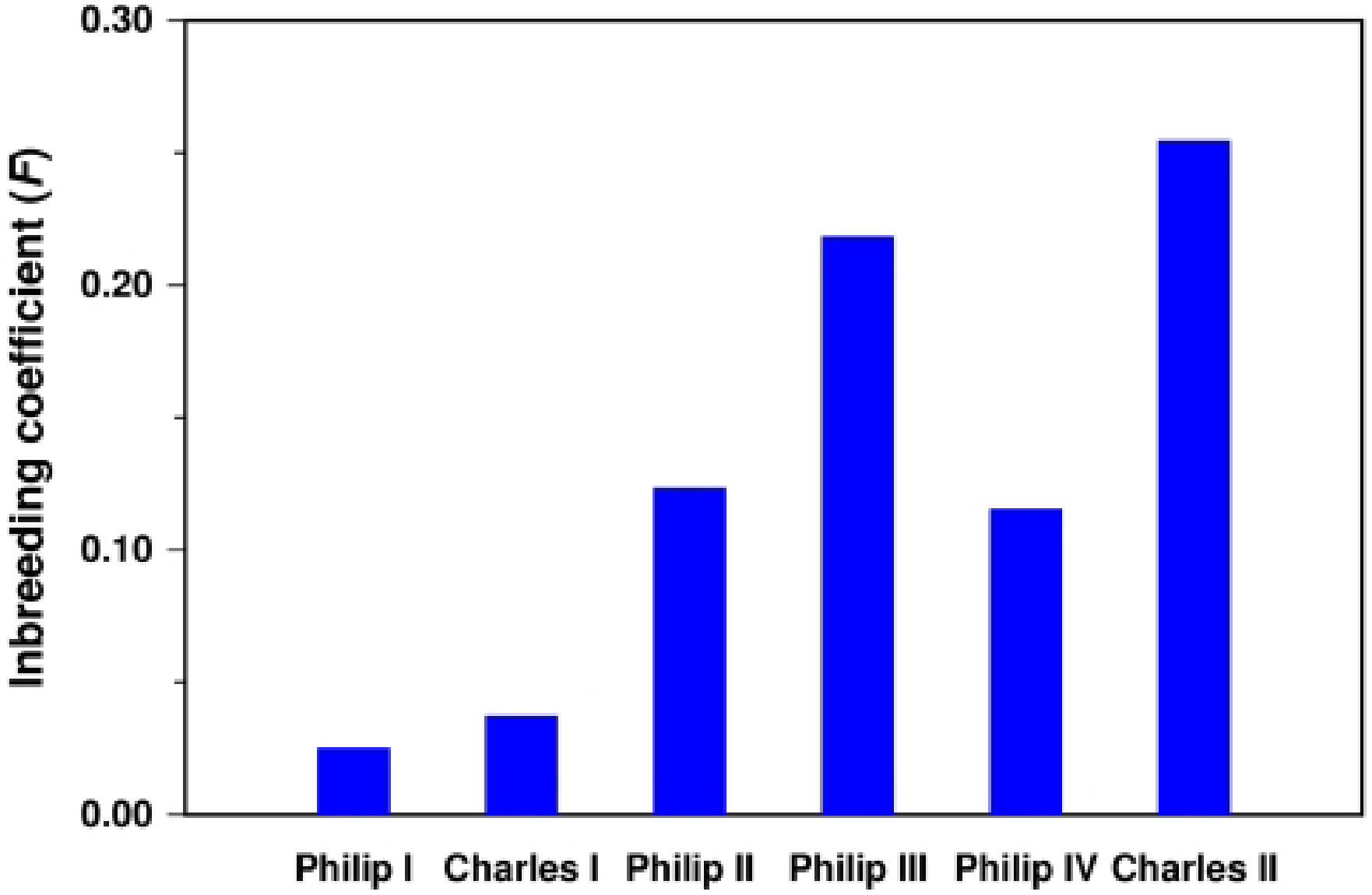
Published online 2009 Apr 15. doi: 10.1371/journal.pone.0005174

A study of 20 generations of Spanish Habsburgs

- Average inbreeding coefficient (from family trees) 0.093
- About 9% of genes have identical versions from mother and father
- For offspring of first cousins, inbreeding coefficient is 0.0625
- For offspring of two third cousins*, inbreeding coefficient is 0.004

- * ‘like England’s Prince Charles’ (Smithsonian Magazine 2019)

- Inbreeding means that recessive mutations that are normally ‘hidden’, get exposed



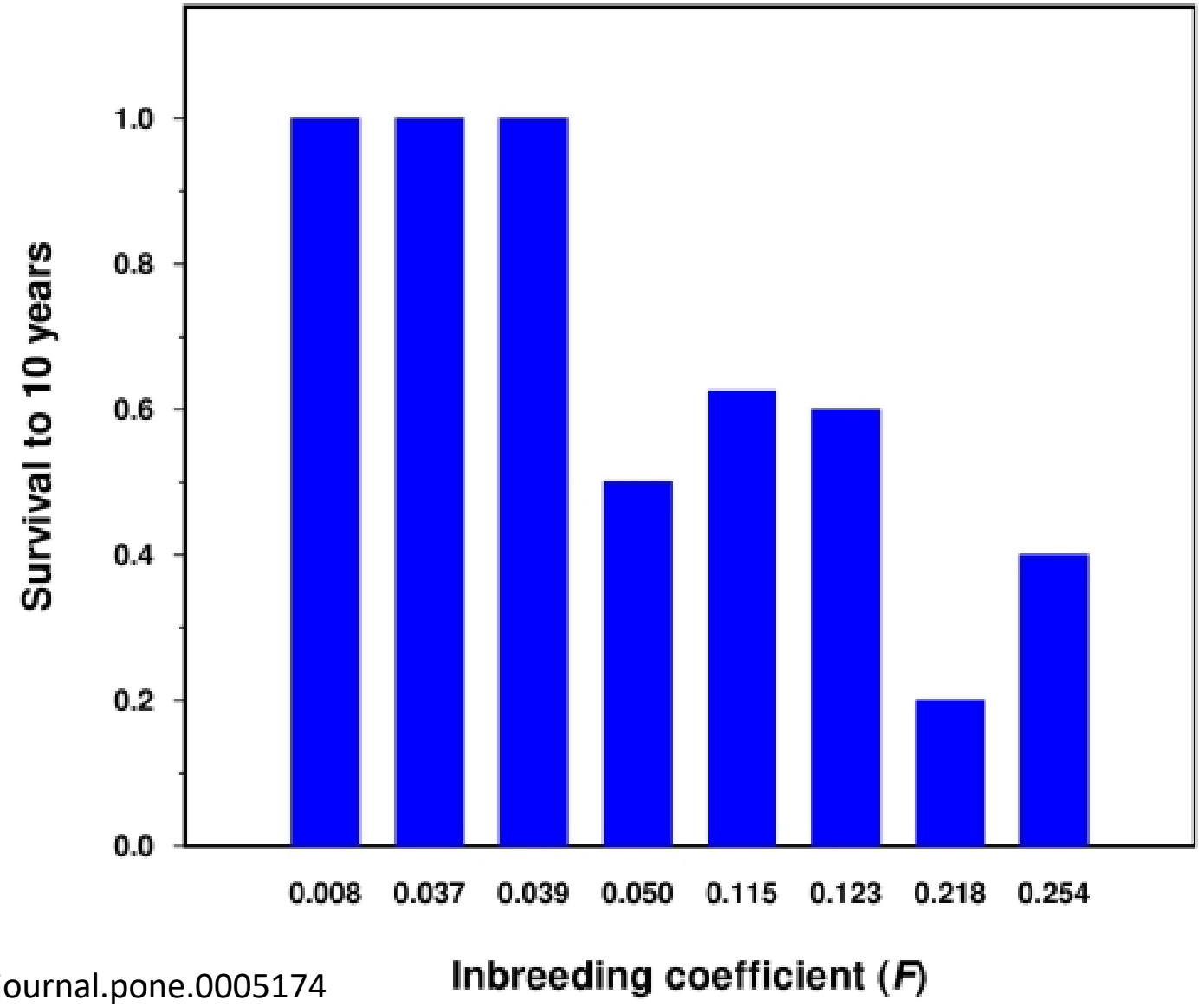
Inbreeding in Spain's Charles II was about 0.25. This is the equivalent to being the offspring of two siblings – due in this case to his parents also being inbred.

Alvarez et al 2009. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0005174

How inbreeding in the Habsburgs affected infant mortality

The accumulation of deleterious mutations in the homozygous recessive form leads to infant mortality as well as other early mortality.

It is possible to plot known infant mortality in Spanish Habsburgs against inbreeding coefficient derived from their pedigrees.



Alvarez et al 2009.
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0005174



“El Hechizado,” or “the bewitched,” as Charles II was dubbed for his **overlarge tongue, epilepsy and other illnesses**, had a whopping inbreeding coefficient of .25, about the same as the offspring of two siblings. (Charles’ mother and father were, in fact, niece and uncle, so this higher value indicates his parents were substantially inbred themselves.) Four years before Charles’ death, British envoy Alexander Stanhope described the king’s Habsburg features in a letter to the Duke of Shrewsbury, writing, “He has a ravenous stomach, and swallows all he eats whole, for **his nether jaw stands so much out, that his two rows of teeth cannot meet**.”

What has been inferred about Charles II of Spain?

Two genes are thought to be responsible for his facial deformities

Two recessive genes are thought to have caused most of his other health issues

- combined pituitary hormone deficiency
- distal renal tubular acidosis

Impotence and sterility (no offspring in his two marriages, the dynasty ended)

Early death at 37 years

Generally regarded as ugly!!

Estimates from human genetics scientists

- Each person carries perhaps 500-1,000 deleterious recessive alleles
- Each person carries 1 or 2 lethal recessive alleles

‘lethal’ meaning causing severe genetic conditions or death before reproductive age, usually when in the homozygous state

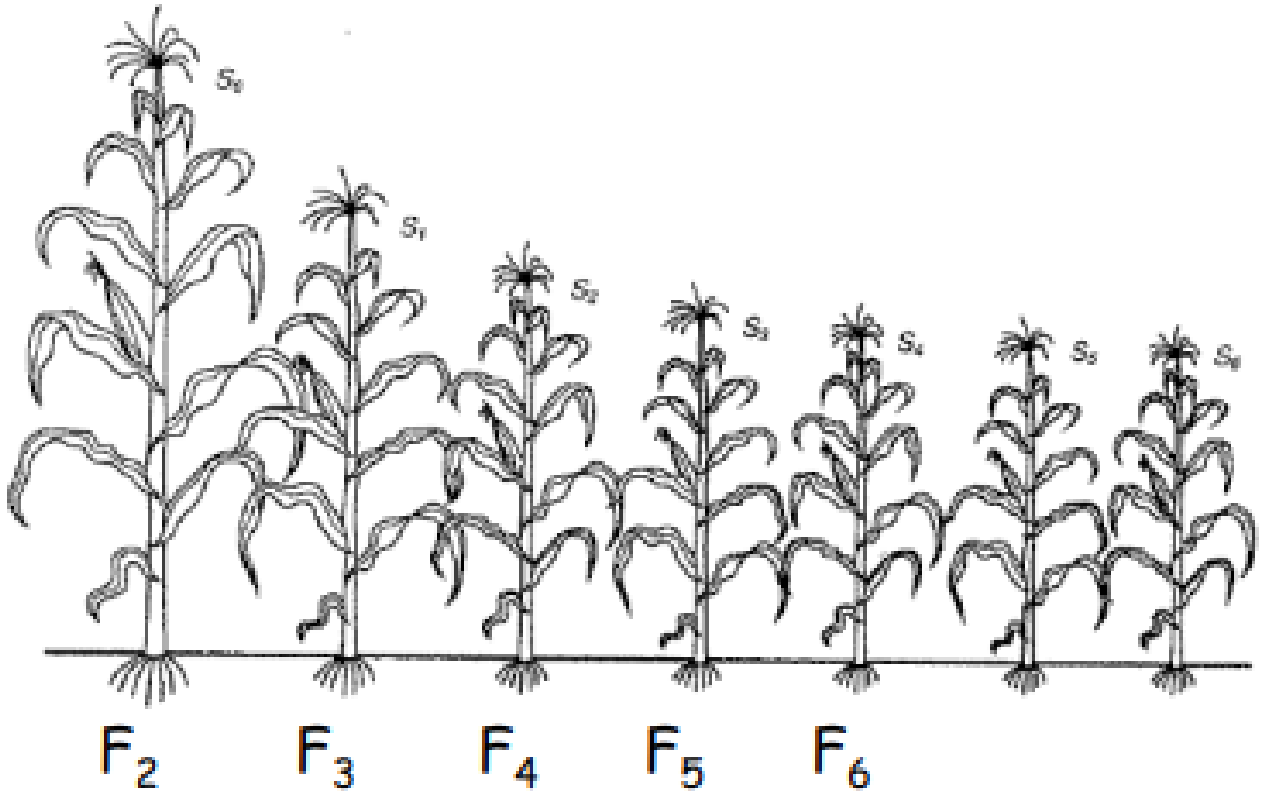
Charles II of Spain may have had many deleterious alleles exposed in his genome

Sung Chun, Justin C. Fay. https://doi.org/10.1371/journal.pgen.1002240

Gao, Ober et al. 2015. https://www.uchicagomedicine.org/forefront/news/humans-carry-one-to-two-lethal-recessive-mutations-on-average-study-estimates

Plants are more flexible

Inbreeding Depression



Example for maize height

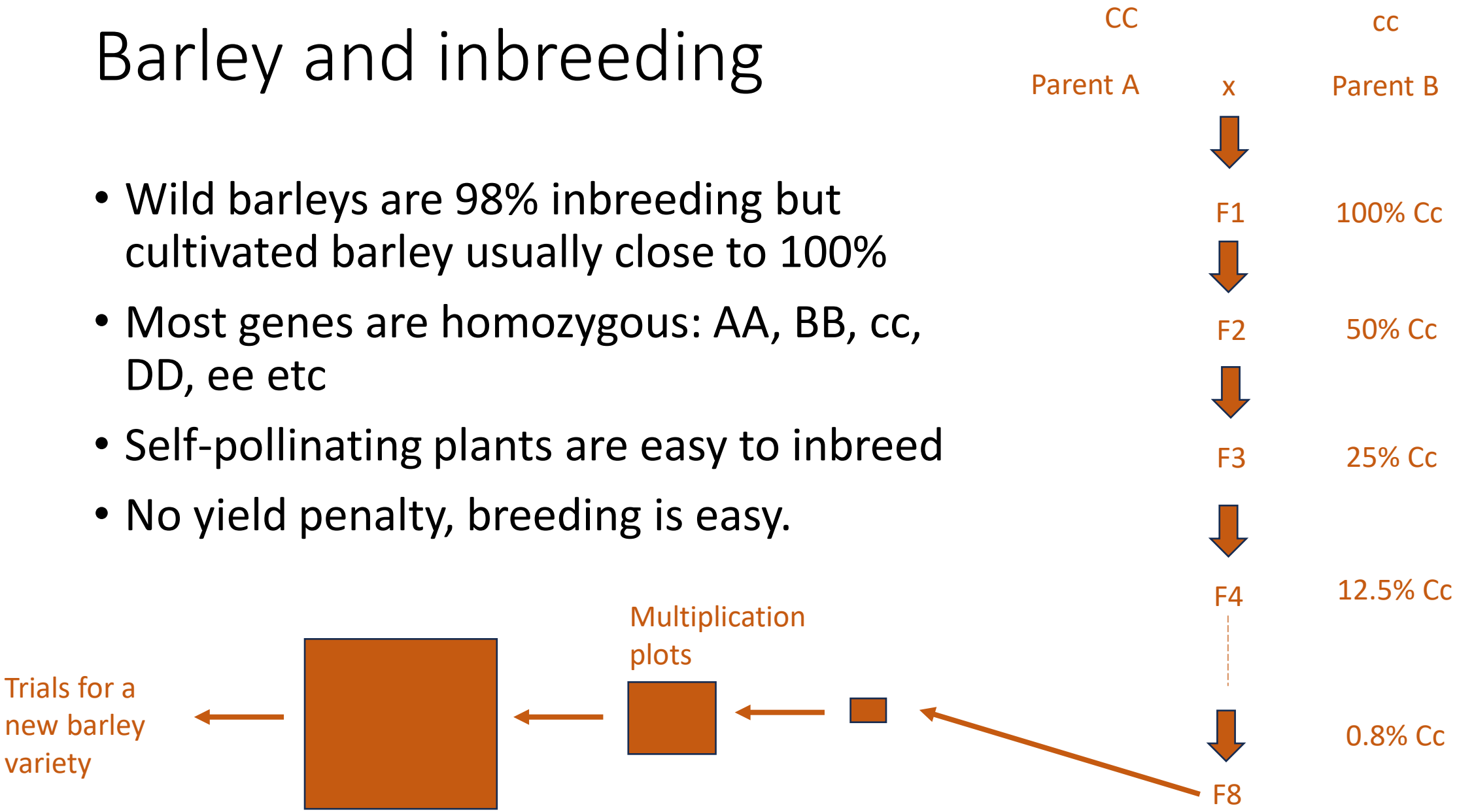
Agricultural Importance of Heterosis

Crosses often show high-parent heterosis, wherein the F_1 not only beats the average of the two parents (mid-parent heterosis), it exceeds the best parent

Crop	% planted as hybrids	% yield advantage	Annual added yield: %	Annual added yield: tons	Annual land savings
Maize	65	15	10	55×10^6	13×10^6 ha
Sorghum	48	40	19	13×10^6	9×10^6 ha
Sunflower	60	50	30	7×10^6	6×10^6 ha
Rice	12	30	4	15×10^6	6×10^6 ha

Barley and inbreeding

- Wild barleys are 98% inbreeding but cultivated barley usually close to 100%
- Most genes are homozygous: AA, BB, cc, DD, ee etc
- Self-pollinating plants are easy to inbreed
- No yield penalty, breeding is easy.



Why are inbred barleys ‘fit’ but inbred maize not?

- In barley, deleterious mutations are naturally selected against heavily
- In maize, deleterious mutations are usually hidden by a dominant allele

Barley is adapted to inbreeding (but retains a small amount of outbreeding)

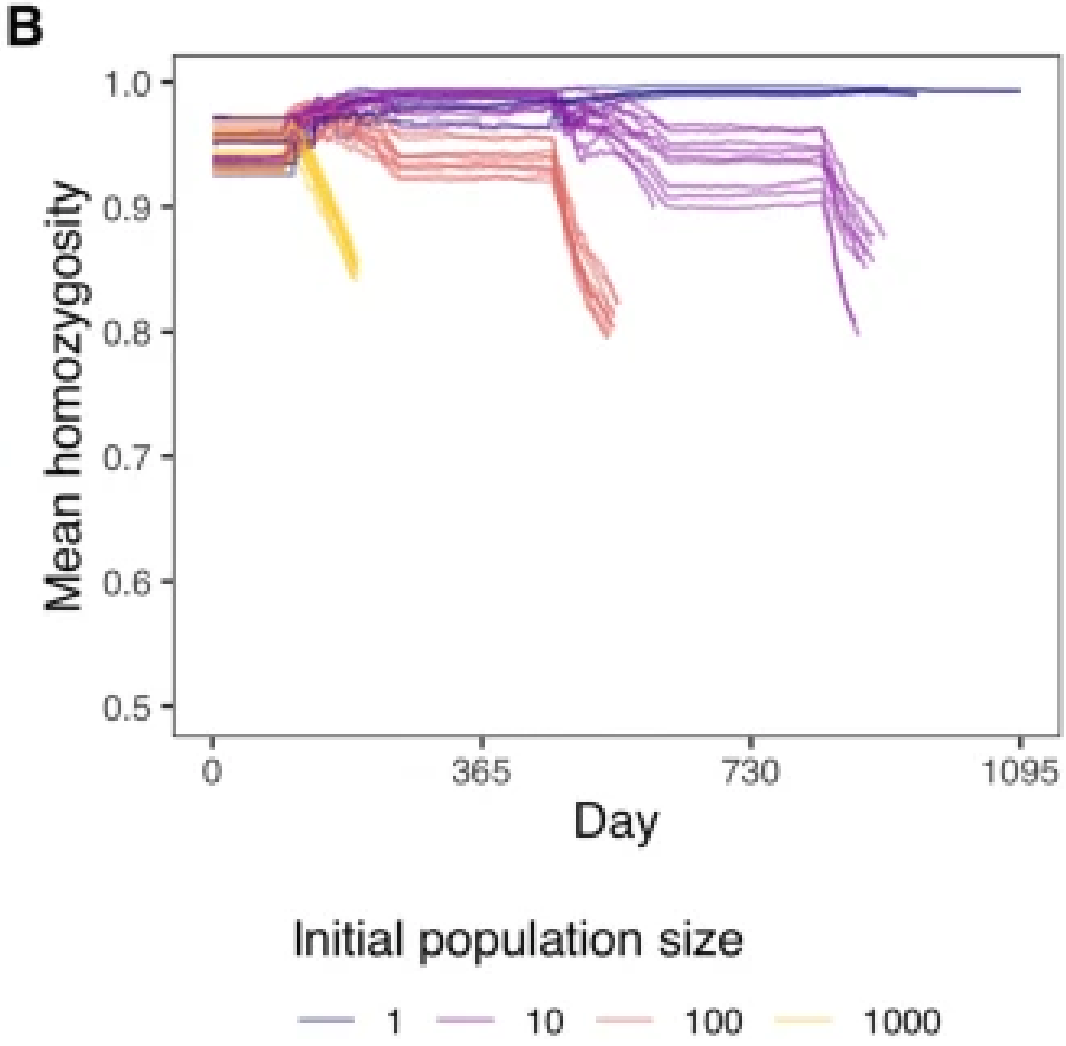
Maize is adapted to outbreeding

Wild barley is less able to shift its population genetics if a new threat appears

Maize can be more adaptable, making new combinations of genes

Inbreeding animals?

- *Varroa* mite*:* mostly brother-sister matings therefore highly inbred
- Occasional crossing (multiple foundresses)
- Crossing is higher late in the season
- Modelled building and spread of acaricide resistance (Faber et al 2021)



Perspectives on In/Outbreeding

- Most plants, insects and other animals are outbreeding
- Animals enforce this by gender (not hermaphrodite), mobility and behaviour
- Mutations accumulate over time, deleterious ones get slowly selected out
- Outbreeding allows deleterious recessive mutations to be hidden
- Inbreeding allows them to be exposed and selected against
- The costs of a switch to inbreeding in most animals (which exposes the ‘genetic load’ carried by the individuals) is huge
- Bees have: haploid males and polyandrous mating plus a genetic sex determination system (using the *csd* gene) relying on high diversity

Apis mellifera bees compared to humans

***Apis mellifera* might carry less of a genetic load compared to humans**

- Haploid males 'expose' deleterious alleles and allow selection against alleles that are *deleterious in males*: purifying selection

***Apis mellifera* needs a diversity of patrilines for colony health and vigour**

- Colonies are composed of patrilines (perhaps 20 or so 'common' ones) and benefit from the genetic diversity of them

***Apis mellifera* has a strong need for genetic diversity at the *csd* locus**

- Sex determination depends on hyper-variability at the *csd* gene

Apis mellifera needs high diversity

1. To avoid issues of genetic load that still occur despite the filter of haploid males

    - this will give at least some protection from ‘bad’ alleles

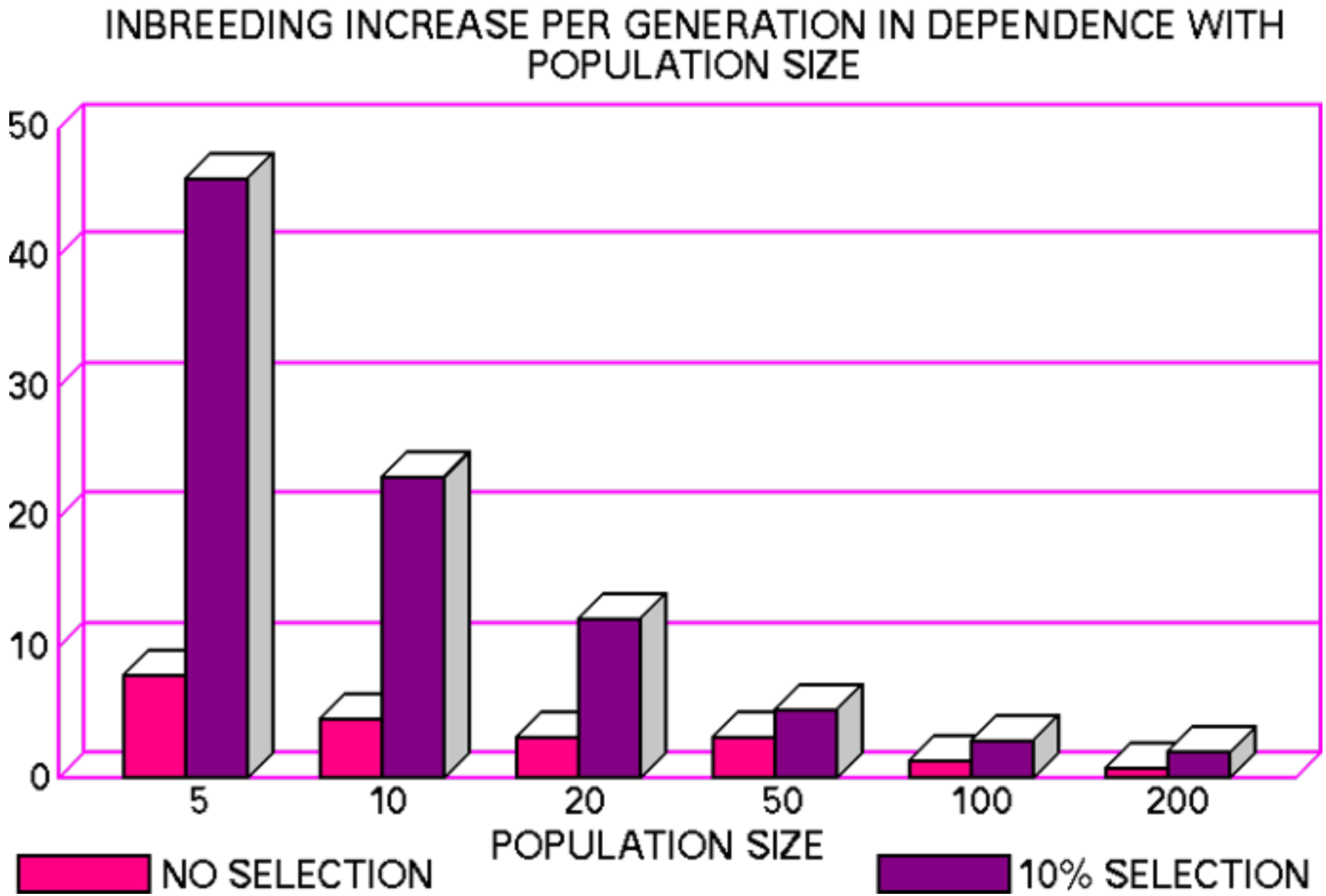
    - issues might be lower than in humans where the equivalent haploid stage is the sperm which is obviously less complex than a drone

2. Maintain genetically diverse patrilines for colony health

    - more and more studies show that diverse patrilines give stronger, healthier colonies

3. Maintain diversity at the *csd* locus to avoid diploid drone issues

    - large inbreeding depression via diploid drones due to *csd*


INBREEDING INCREASE PER GENERATION IN DEPENDENCE WITH POPULATION SIZE
50
40
30
20
10
0
5
10
20
50
100
200
POPULATION SIZE
NO SELECTION
10% SELECTION

Inbreeding has strong effects via the *csd* gene

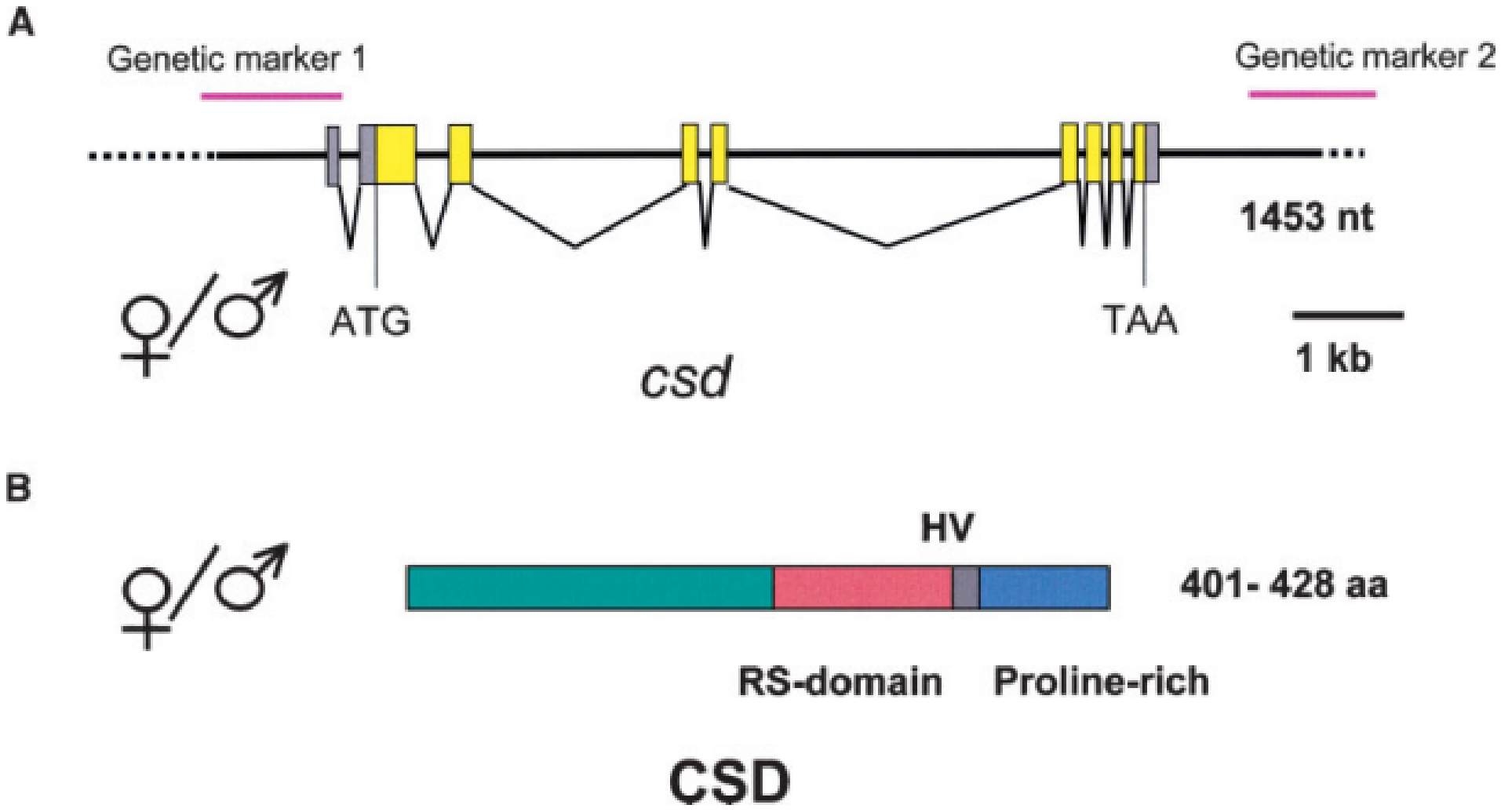
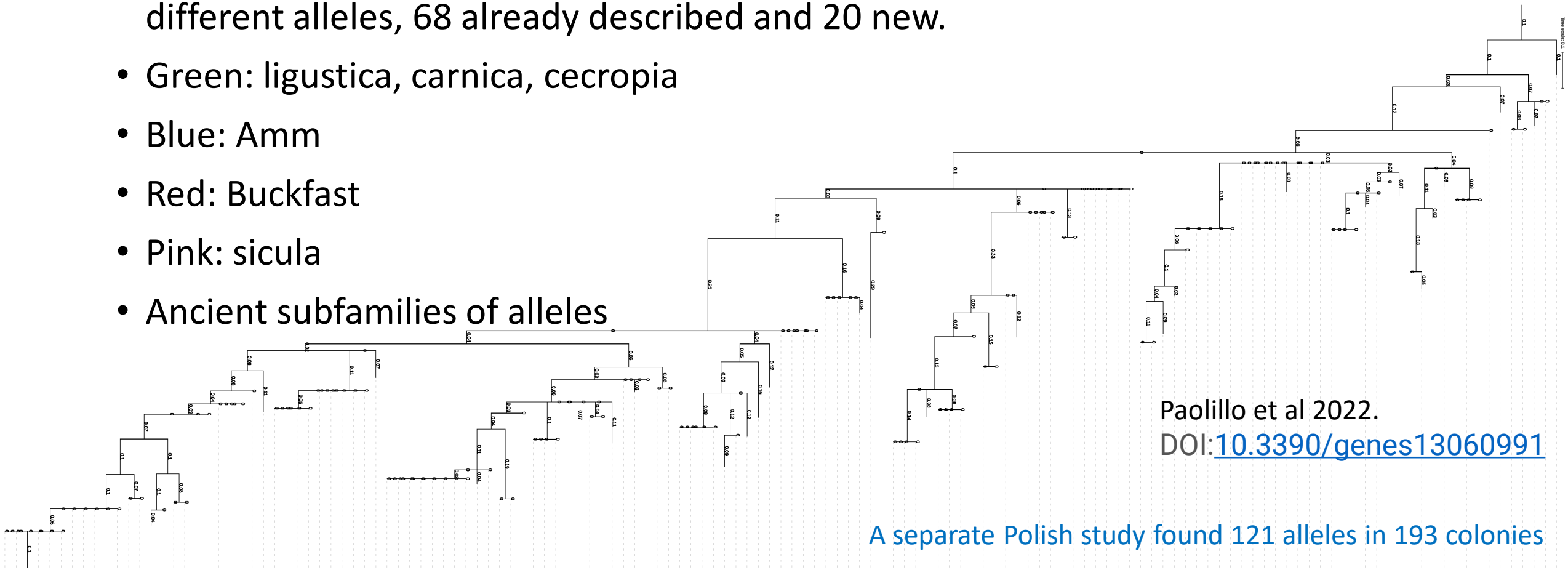


Figure 3. Schematic presentation of the genomic organization and proposed protein structure of *csd*. **A:** Genomic region encompassing *csd* and the two closely linked genetic markers (marker 1 and 2). The genetic markers 1 and 2 are always heterozygous in females; this identifies the sex-determining locus. Exons are indicated by boxes and the deduced ORF is marked in yellow. The estimate of the transcript is indicated in nucleotides (nt), which represents just one allelic variant. No sex-specific differences were found among the transcripts. Predicted translational start and stop sites are indicated. **B:** The schematic presentation of the predicted domain structure of *csd*. The region rich in arginine/serine (RS domain) is marked in red, the hypervariable region that has different number of repeats in various alleles is shown in grey (HV) and the proline-rich region is marked in blue. The number of amino acids of the CSD protein varies between alleles as indicated.[55]

Beye 2004. DOI: 10.1002/bies.20098

Paolillo et al 2022: *CSD* alleles in Italy

- Sequencing of the Hypervariable region of the csd gene in 125 workers gave 88 different alleles, 68 already described and 20 new.
- Green: ligustica, carnica, cecropia
- Blue: Amm
- Red: Buckfast
- Pink: sicula
- Ancient subfamilies of alleles



Paolillo et al 2022.
DOI:10.3390/genes13060991

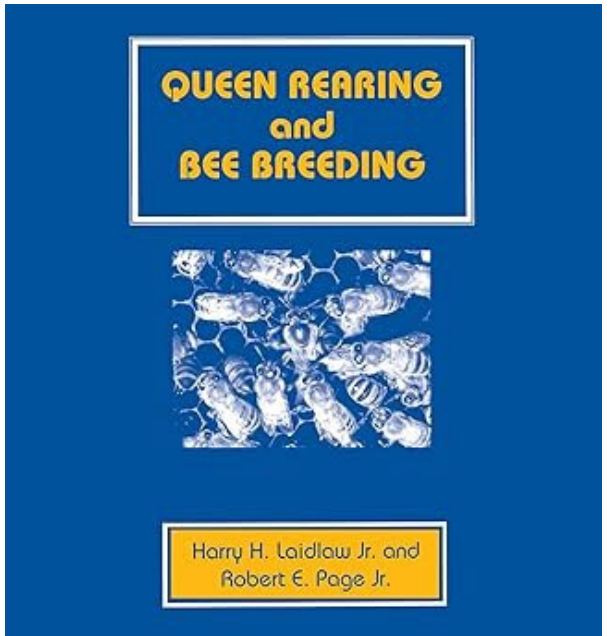
A separate Polish study found 121 alleles in 193 colonies

CSD gene diversity

- Allele diversity at *csd* is very high because heterozygosity is fit and homozygosity is disastrous
- Various estimates show very high numbers of alleles in regional populations – populations must need that diversity for population health
- Modelling (30 years ago) shows that the minimum population size for genetic health in *Apis mellifera* is high, just for this one gene
- Traditionally ~50 individual queens/colonies seen as a minimum, Sue Cobey now uses more, perhaps 200 stocks
- New insights into molecular genetics hints that the larger populations may be optimal

Laidlaw and Page modelled inbreeding at *csd*

- Written 1996
- ‘populations of honey bees estimated to have between 6 and 17 different sex alleles’
- Closed population breeding assumed to need to maintain >85% brood viability, and to last 20 years of one generation per year
- Simulations suggest that 35-50 queens were needed to maintain reasonable brood viability
- Full mixing was assumed.



Would their simulations apply today?

- Estimates of numbers of *csd* alleles maintained in populations have climbed 10 fold
- The numbers of matings thought to take place have also increased
- 15% brood loss may be considered high now

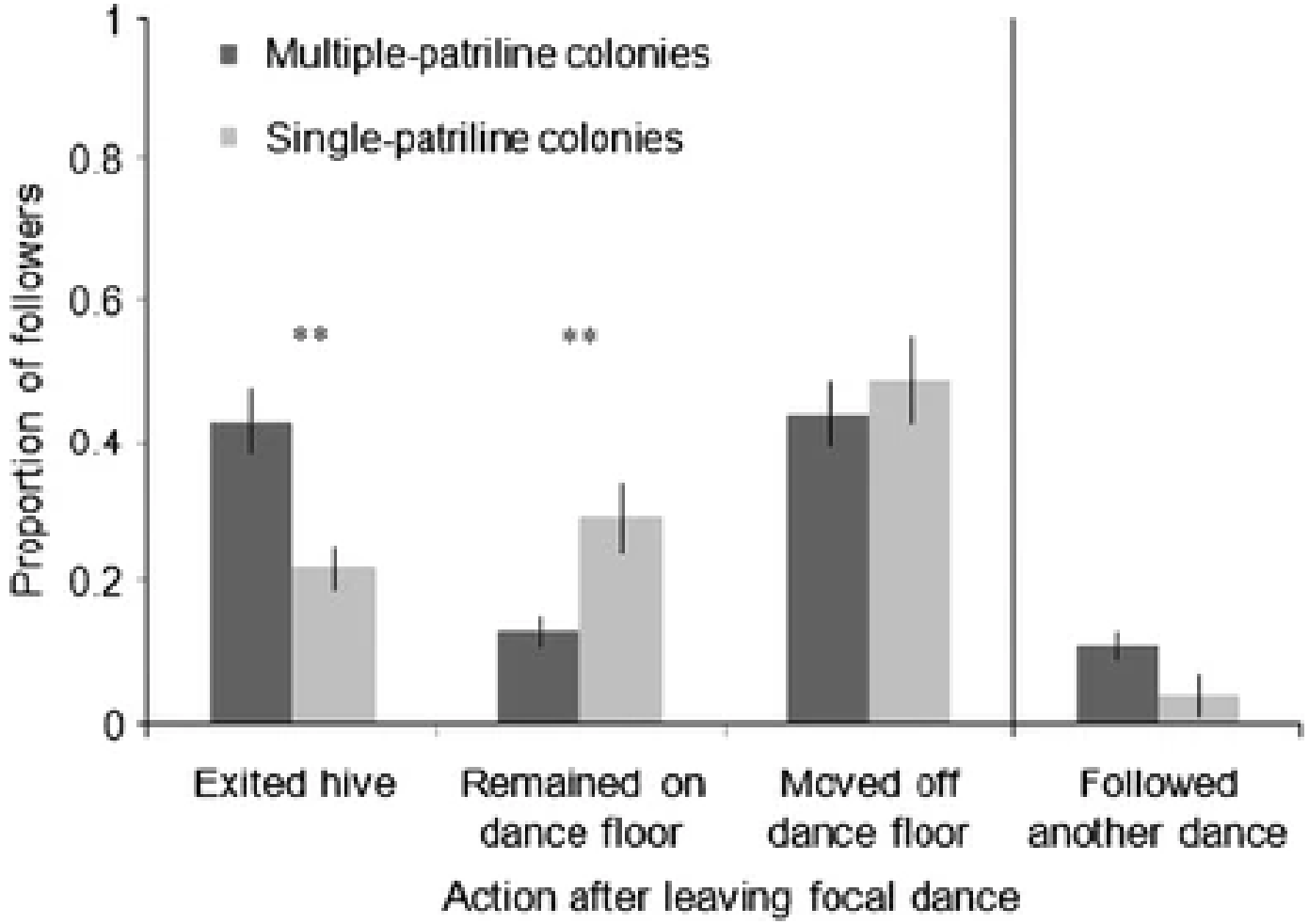
- Does the overall genetic load also influence colony vigour?

- In that light, Sue Cobey now using larger population sizes seems appropriate

Other inbreeding effects via patriline diversity

- single drone or multiple drone inseminations

- drone diversity affects worker activity and disease resistance



THE ROYAL SOCIETY

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Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth

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Multiple mating by social insect queens increases the genetic diversity among colony members, thereby reducing intracolony relatedness and lowering the potential inclusive fitness gains of altruistic workers. Increased genetic diversity may be adaptive, however, by reducing the prevalence of disease within a nest. Honeybees, whose queens have the highest levels of multiple mating among social insects, were investigated to determine whether genetic variation helps to prevent chronic infections. I instrumentally inseminated honeybee queens with semen that was either genetically similar (from one male) or genetically diverse (from multiple males), and then inoculated their colonies with spores of *Ascosphaera apis*, a fungal pathogen that kills developing brood. I show that genetically diverse colonies had a lower variance in disease prevalence than genetically similar colonies, which suggests that genetic diversity may benefit colonies by preventing severe infections.

Keywords: disease; genetic diversity; honeybees; polyandry

Proc Biol Sci. 2007 Jan 7; 274(1606): 67–72.
Published online 2006 Sep 26. doi: 10.1098/rspb.2006.3702
PMCID: PMC1679871
PMID: 17015336

Queen promiscuity lowers disease within honeybee colonies

Thomas D Seeley[1,*] and David R Tarpy[2]

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Abstract

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Most species of social insects have singly mated queens, but in some species each queen mates with numerous males to create a colony with a genetically diverse worker force. The adaptive significance of polyandry by social insect queens remains an evolutionary puzzle. Using the honeybee (*Apis mellifera*), we tested the hypothesis that polyandry improves a colony's resistance to disease. We established colonies headed by queens that had been artificially inseminated by either one or 10 drones. Later, we inoculated these colonies with spores of *Paenibacillus larvae*, the bacterium that causes a highly virulent disease of honeybee larvae (American foulbrood). We found that, on average, colonies headed by multiple-drone inseminated queens had markedly lower disease intensity and higher colony strength at the end of the summer relative to colonies headed by single-drone inseminated queens. These findings support the hypothesis that polyandry by social insect queens is an adaptation to counter disease within their colonies.

Proc Biol Sci
Proc Biol Sci

There are three ways inbreeding affects honeybees

1. Exposure of ‘genetic load’ via the encouragement of homogygosity for deleterious alleles (essentially ‘inbreeding depression’)
2. Low diversity of patrilines
3. Insufficient diversity at the *csd* gene

What does this all mean for bee conservation

- Island or other closed populations are always at risk of inbreeding
- Any attempt at breeding small ‘closed’ populations is doomed to failure in a small number of generations
- It is urgent to mix isolated populations that still carry useful genetic diversity, if they meet other criteria for breeding
- Organisations like SNHBS and any small breeding groups allied to the organisation have an absolute need to work together and share breeding lines regularly

How to assess inbreeding in *Apis mellifera*?

Molecular tool exist to assess inbreeding

- 'runs of homozygosity' – large sets of SNPs aligned along chromosomes which can be allocated into different states
- Simple Sequence Repeats or Microsatellites – these are usually multiallelic assays
- Sequencing of single genes (*csd* would be a good example)

Beekeepers can also assess inbreeding

- Proportion of worker larvae removed at a v early stage (policing of diploid drone larvae) or persisting to give domed cappings – this gives an indication of a paucity of variation at the *csd* gene
- General vigour may also indicate level of inbreeding

Further thoughts

We are still guessing at

- the diversity in sub-populations or subspecies
- the levels of inbreeding which compromise genetic health
- population sizes needed for sustainability over known time periods

There is an urgent need to have appropriate

- scientific input to explore genetic diversity
- support to preserve remaining Scottish Amm lines
- support the finding and enable the crossing of remaining diversity