Multiple hybrid origins of diploid and polyploid asexual lineages of the brine shrimp Artemia

Nicolas Rode

INRA SCIENCE & IMPACT

Centre for Biology and Management of Populations
CBGP
Centre de Biologie pour la Gestion des Populations

CBGP, May 5 2020

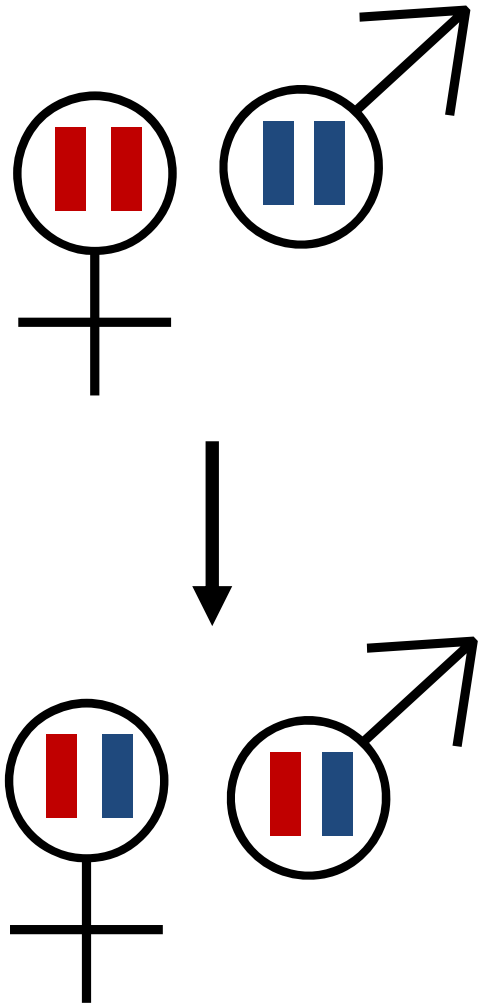
Acknowledgements

Thomas Lenormand

Roula Zahab

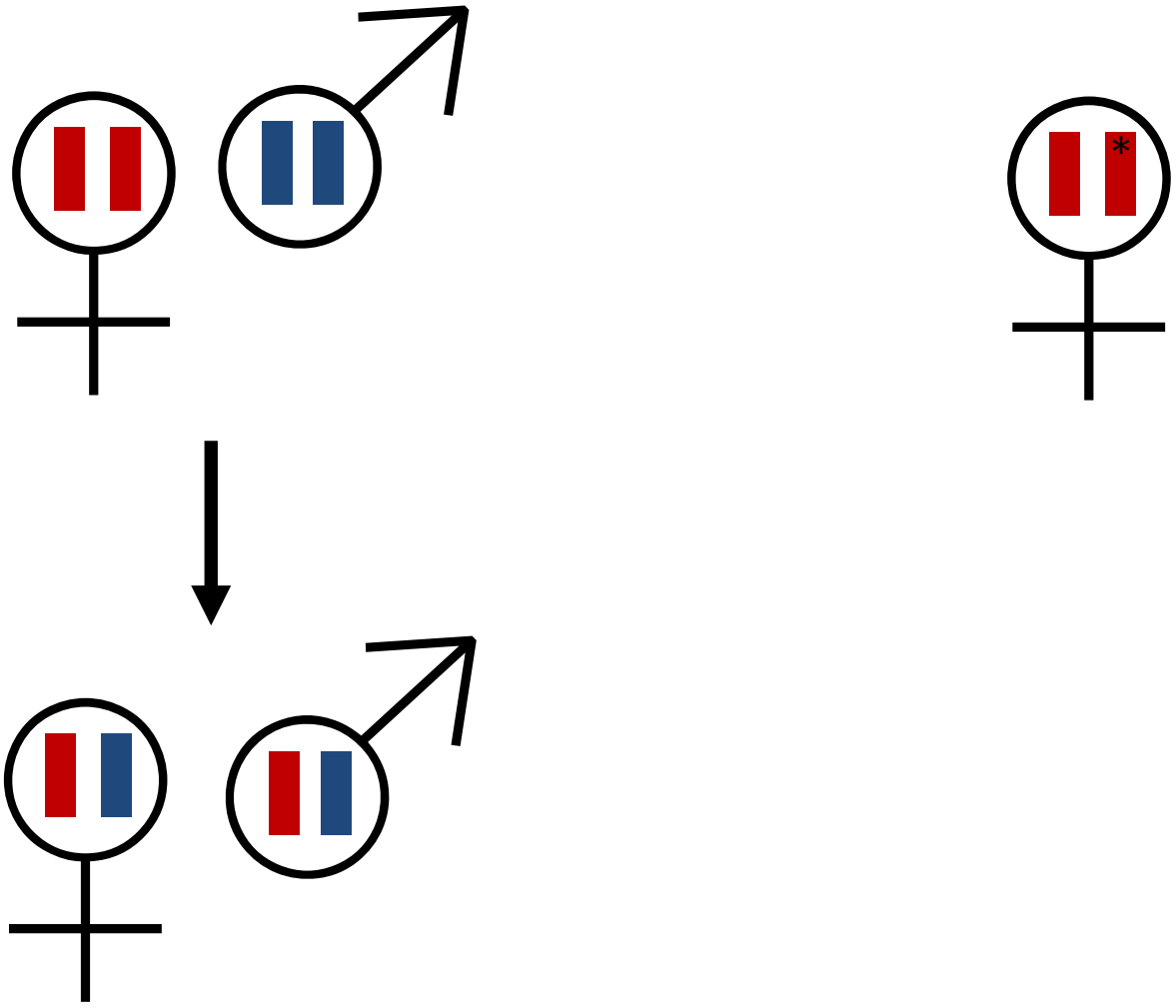
Elodie Flaven

The two-fold cost of sex



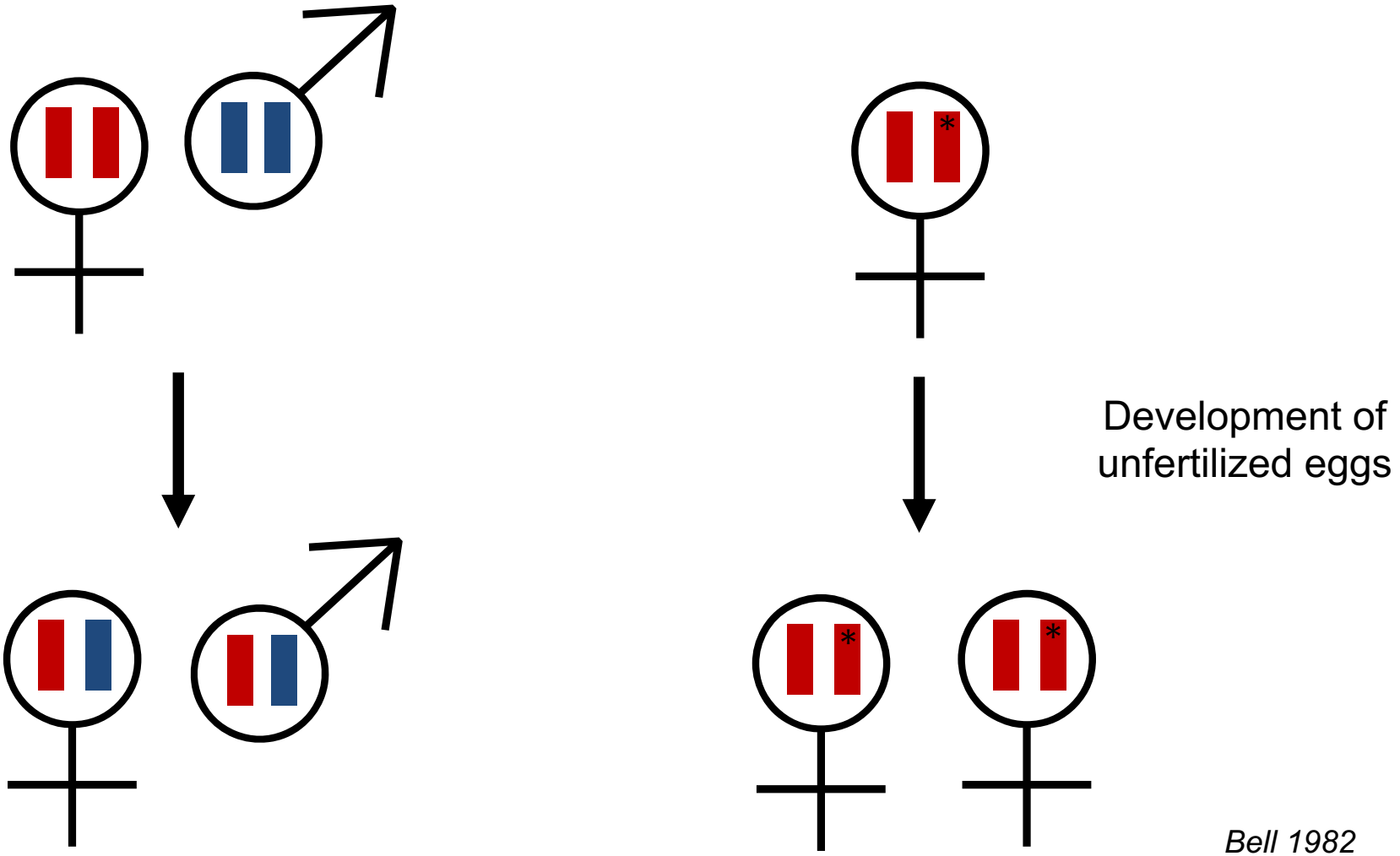
Bell 1982

The two-fold cost of sex



Bell 1982

The two-fold cost of sex



Bell 1982

Other potential costs

Attracting a mate

Other potential costs

Attracting a mate

Predation

Other potential costs

Attracting a mate

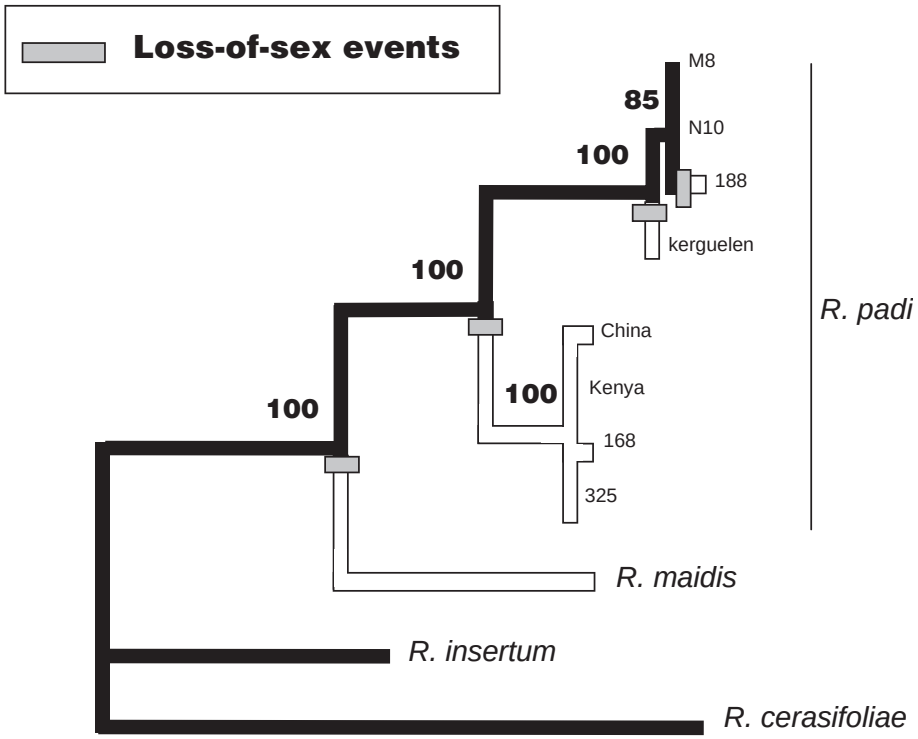
Predation

Sexually transmited diseases

Puzzle of the phylogenetic distribution of asexuals

Exclusive asexuality:
~0.1% in animals (Vrijenhoek 1998)
~1% in plants (Whitton et al 2008)

Tip of phylogenies



Rhopalosiphum padi

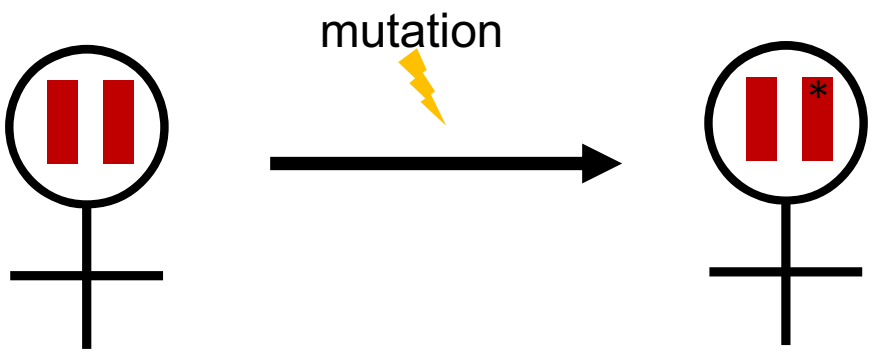
Delmotte et al 2001

Current hypotheses

- short-term benefits (eg no cost of sex, increased colonization ability)
- long-term costs (eg accumulation of deleterious mutations, low adaptive potential)

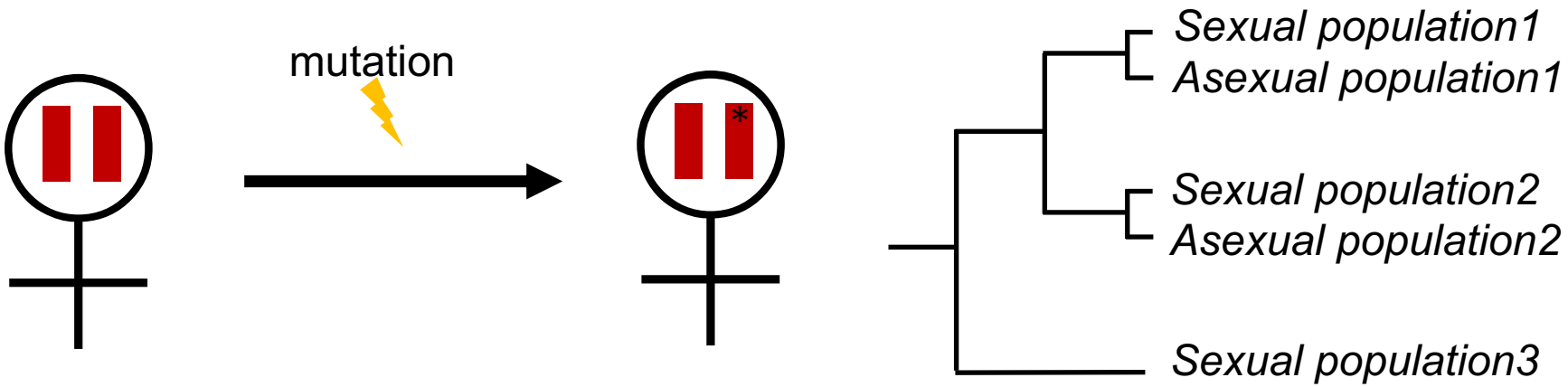
Current hypotheses

- short-term benefits (eg no cost of sex, increased colonization ability)
- long-term costs (eg accumulation of deleterious mutations, low adaptive potential)



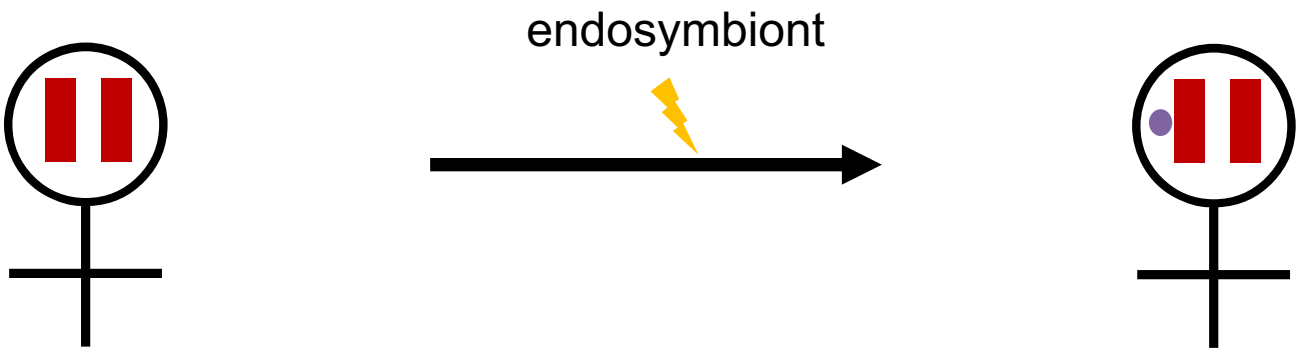
Current hypotheses

- short-term benefits (eg no cost of sex, increased colonization ability)
- long-term costs (eg accumulation of deleterious mutations, low adaptive potential)



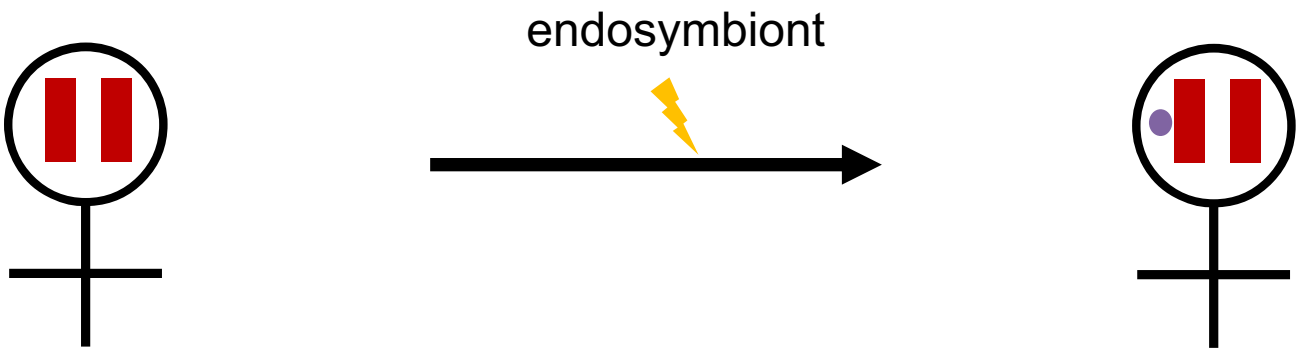
Infectious origin of asexuality

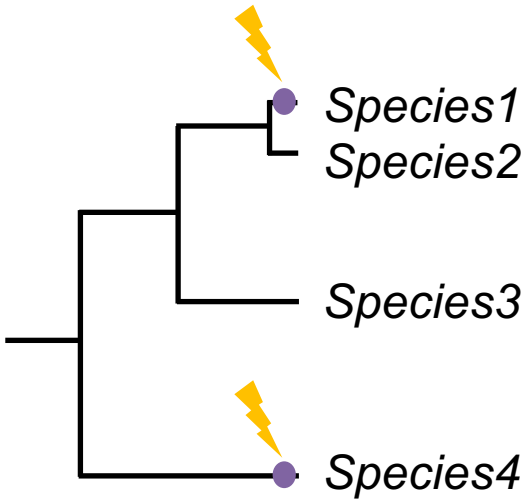
(e.g. hymenoptera, thrips, mites)



Infectious origin of asexuality

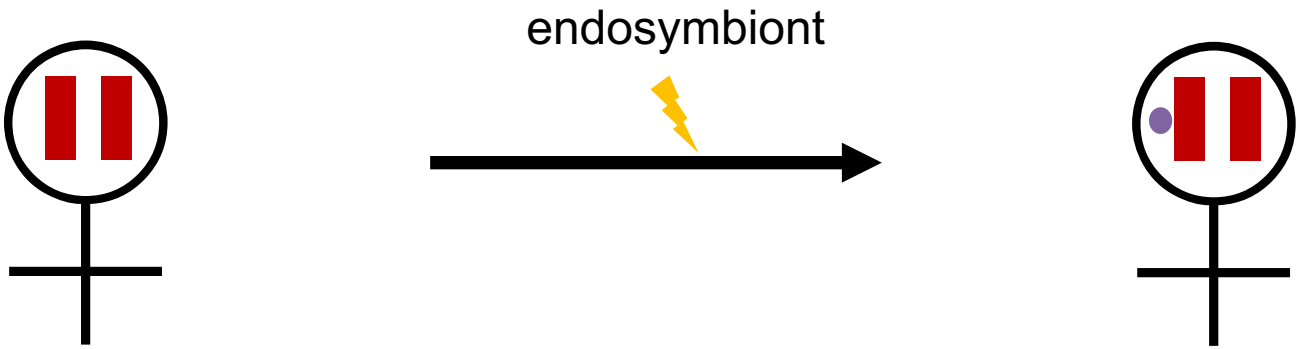
(e.g. hymenoptera, thrips, mites)

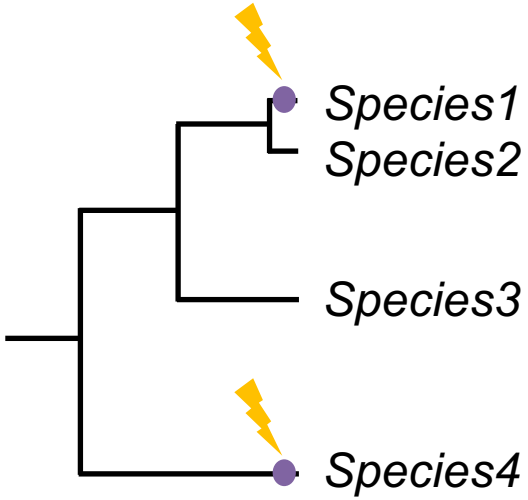




Infectious origin of asexuality

(e.g. hymenoptera, thrips, mites)

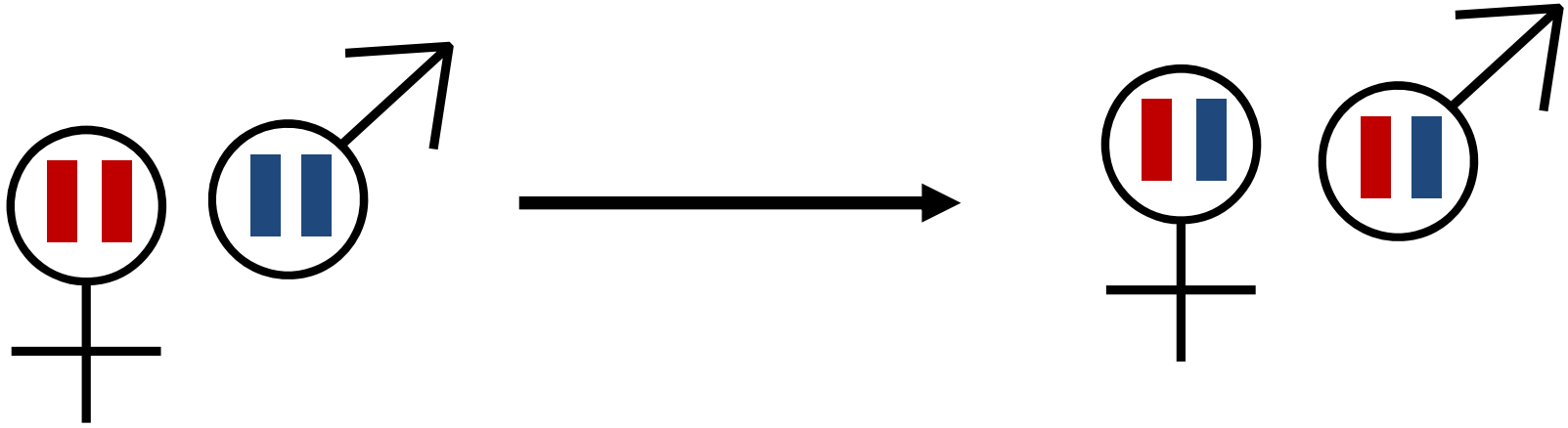




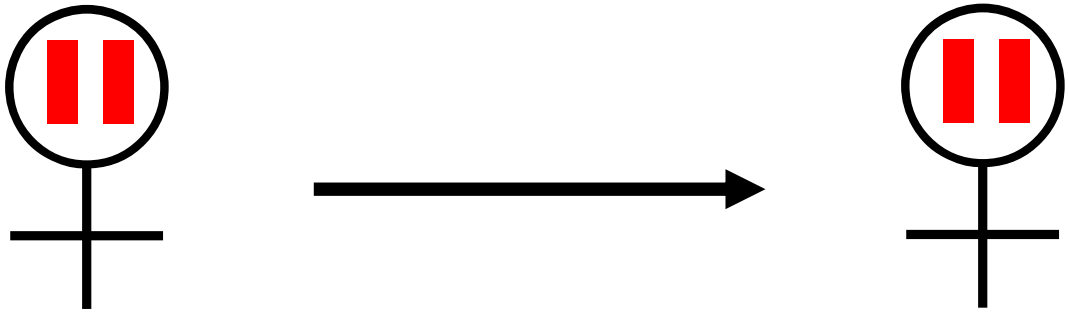
Rate of evolution of parthenogenesis-induction?
Rates of vertical or horizontal infection?
Reversion possible?
Host specialization of the symbiont?

Contagious origin of asexuality

(e.g. aphids, daphnia)



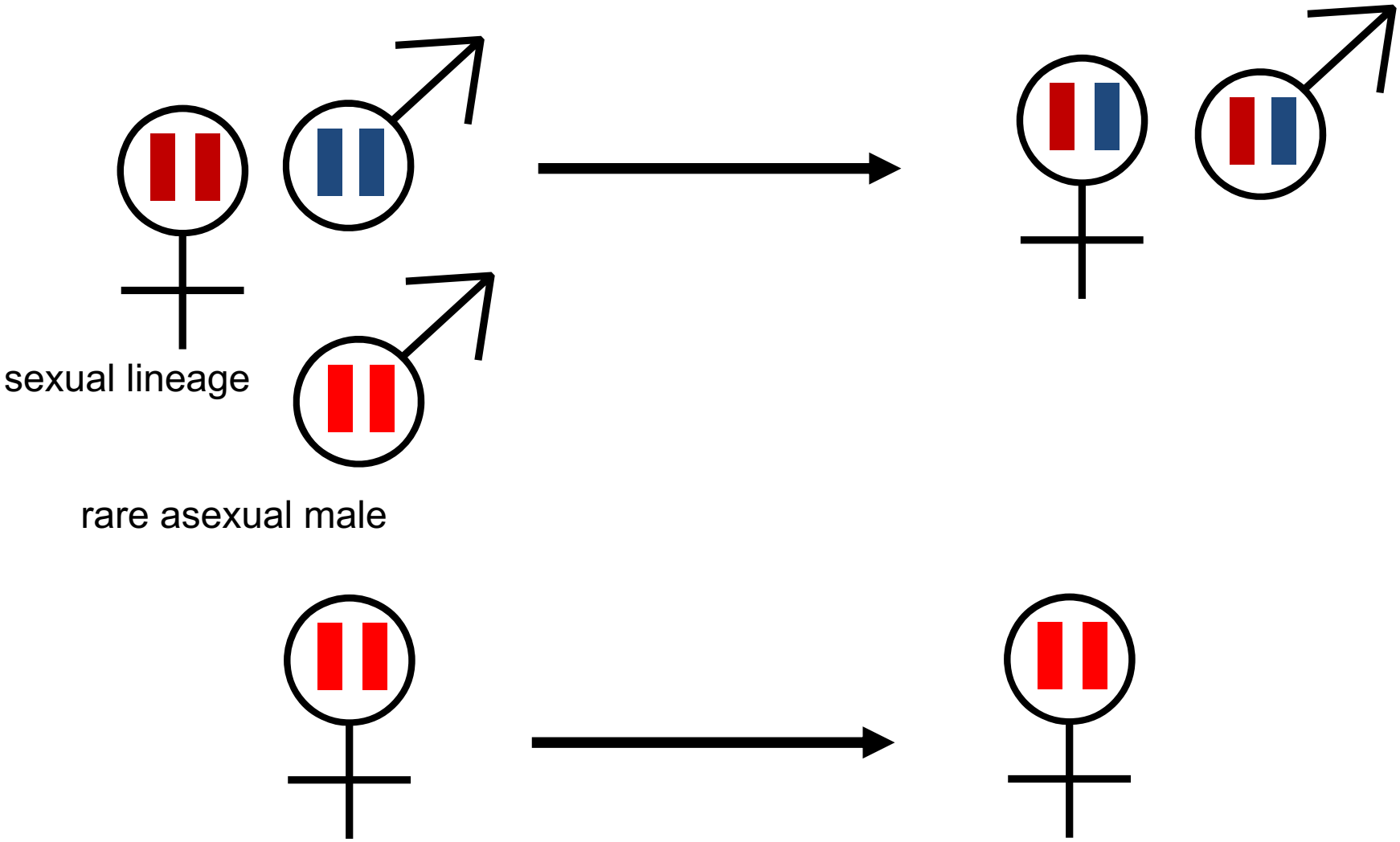
sexual lineage



asexual lineage

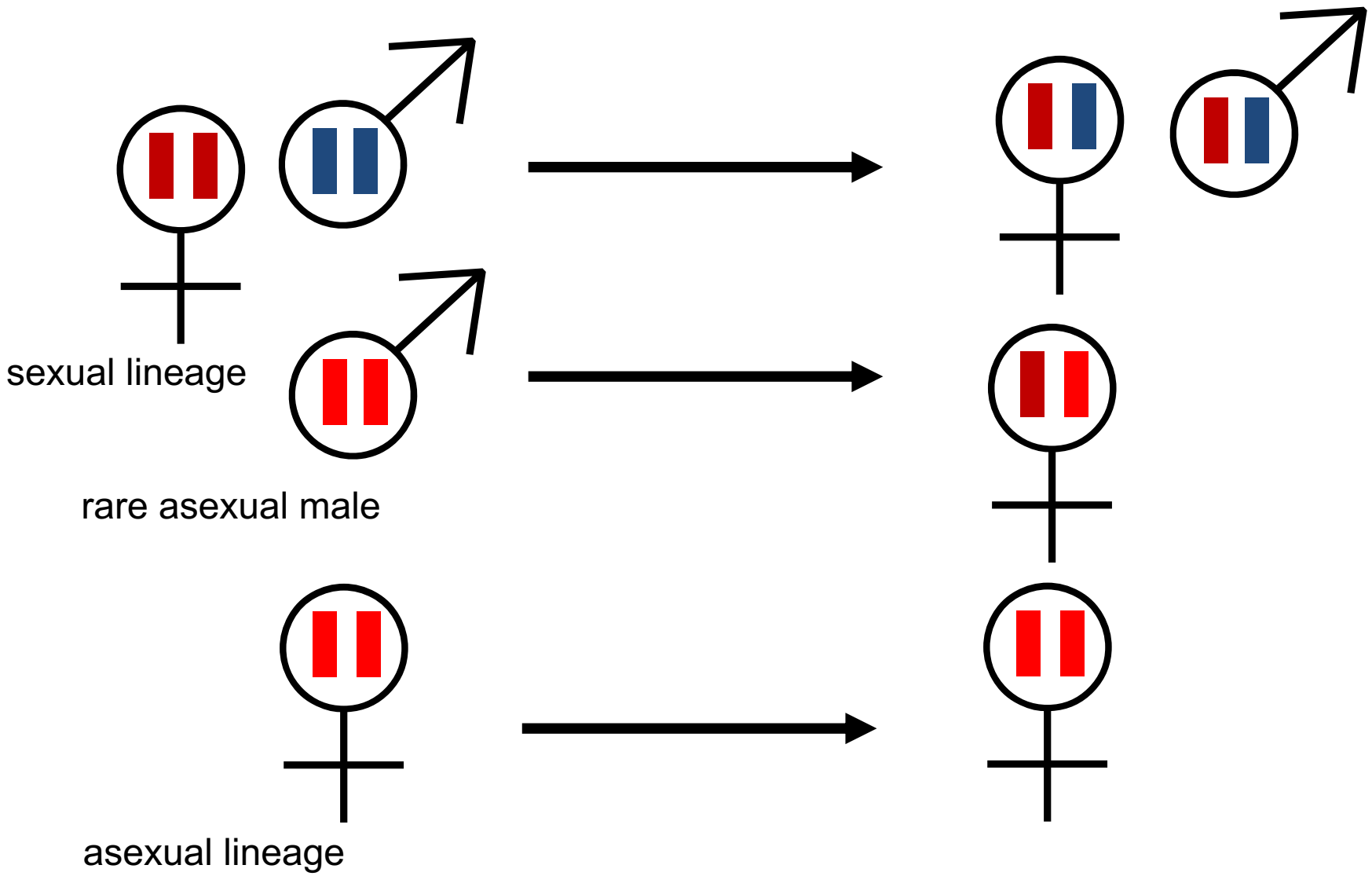
Contagious origin of asexuality

(e.g. aphids, daphnia)



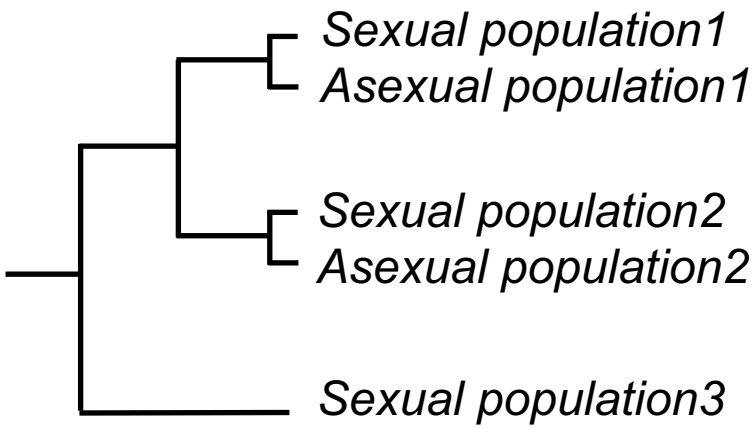
Contagious origin of asexuality

(e.g. aphids, daphnia)



Contagious origin of asexuality

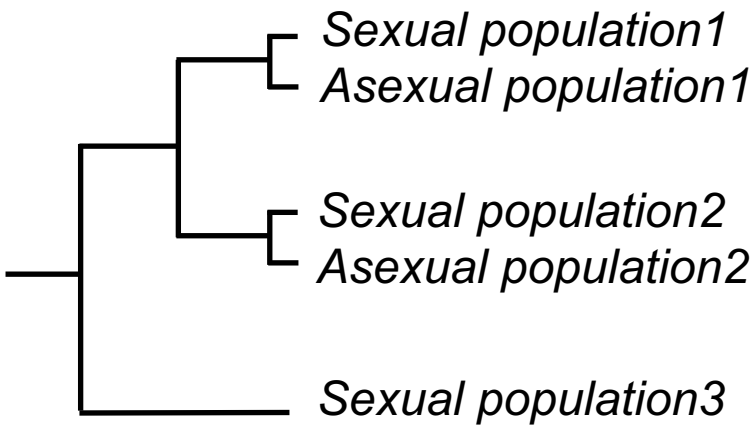
(e.g. aphids, daphnia)



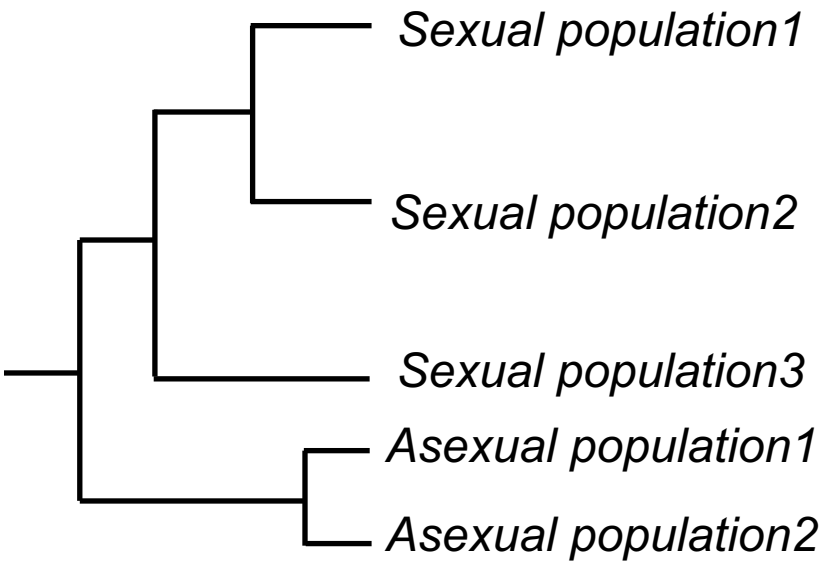
random locus

Contagious origin of asexuality

(e.g. aphids, daphnia)



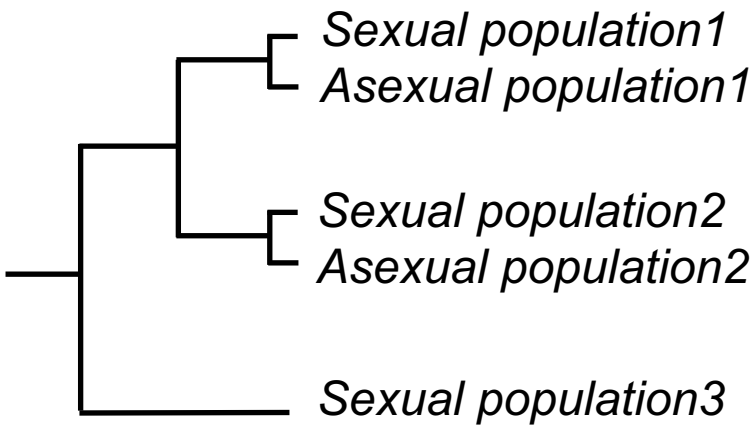
random locus



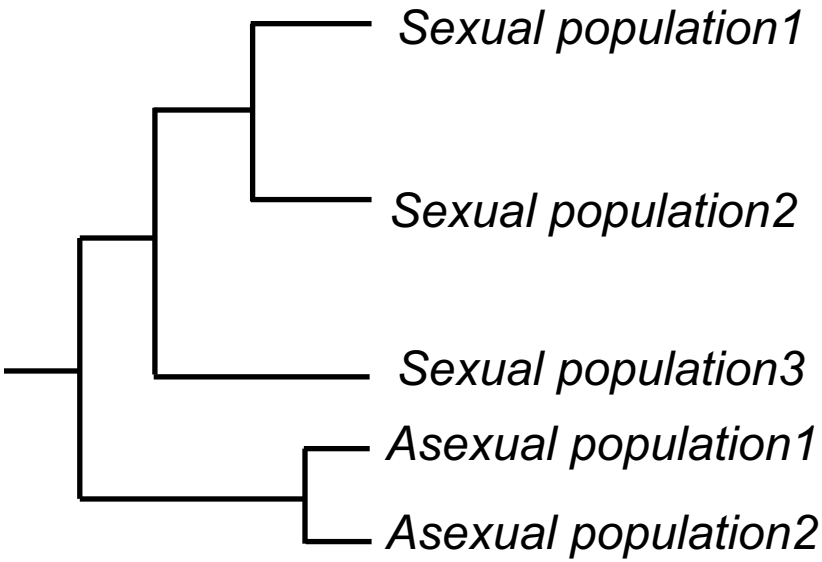
asexuality determining locus

Contagious origin of asexuality

(e.g. aphids, daphnia)



random locus

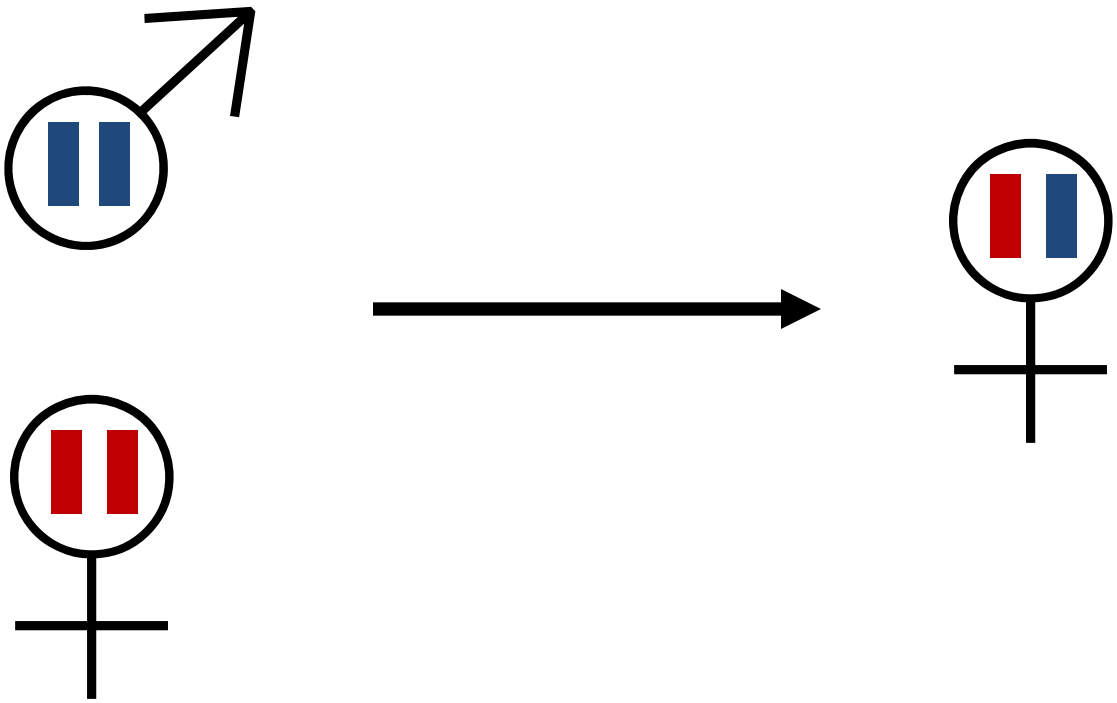


asexuality determining locus

Rate of production of functional rare males?
Rate of hybridization with sexual relatives?
Fitness of hybrids?
F1 reproduce sexually and/or asexually?
Genetic basis of asexuality?

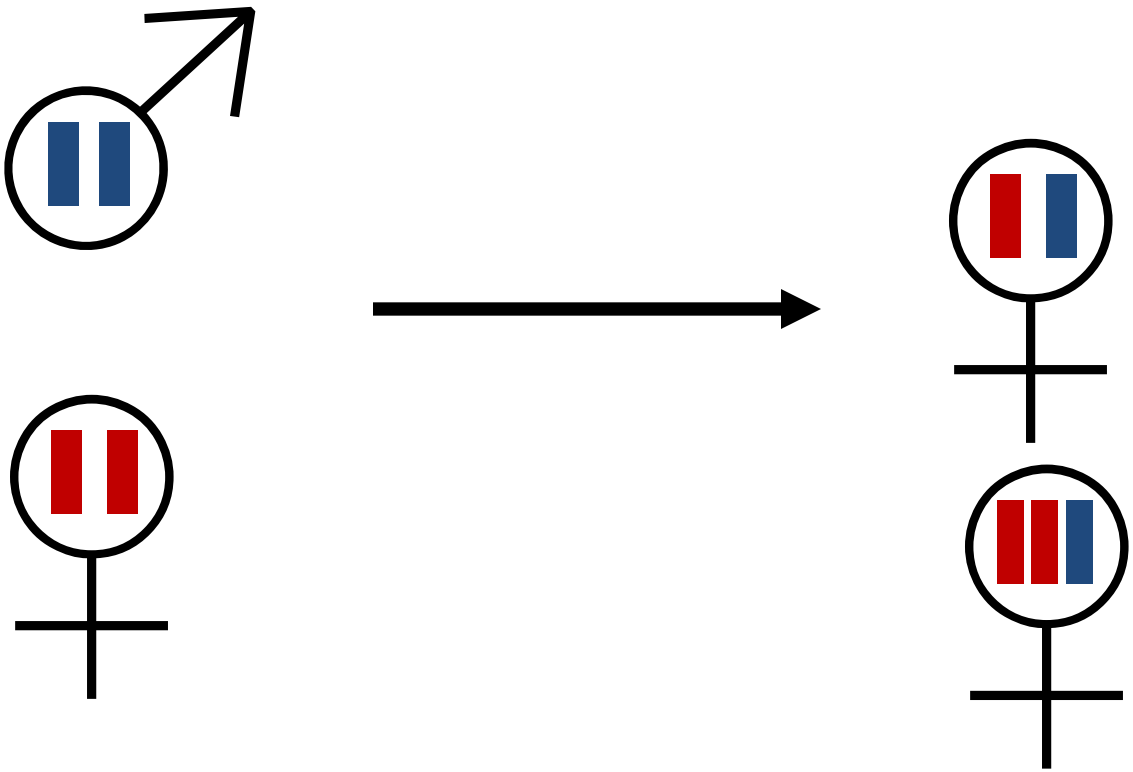
Hybrid origin of asexuality

(e.g. weevils, stick insects, grasshopers)



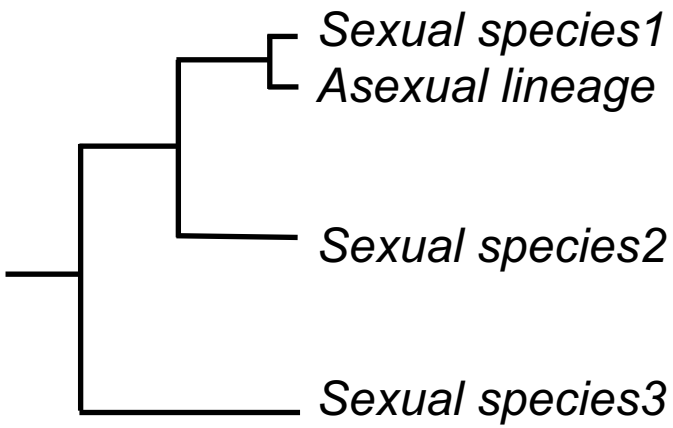
Hybrid origin of asexuality

(e.g. weevils, stick insects, grasshopers)



Hybrid origin of asexuality

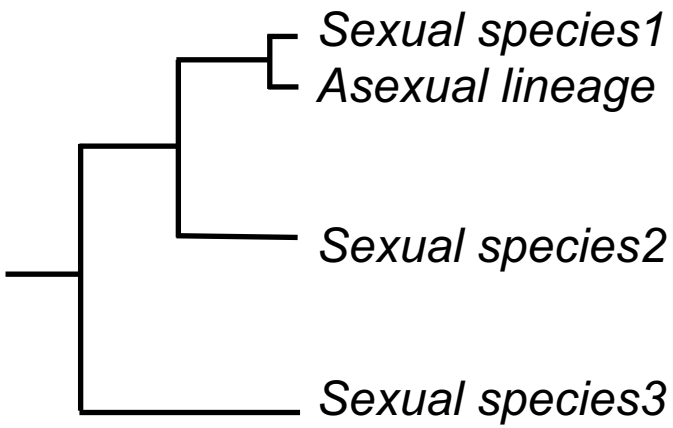
(e.g. weevils, stick insects, grasshopers)



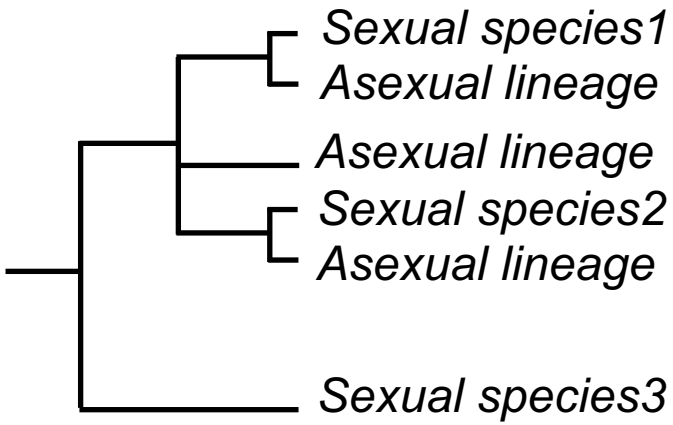
mitochondrial locus

Hybrid origin of asexuality

(e.g. weevils, stick insects, grasshopers)



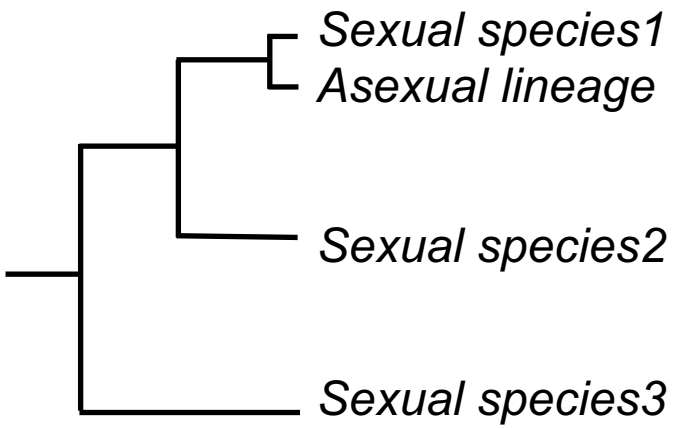
mitochondrial locus



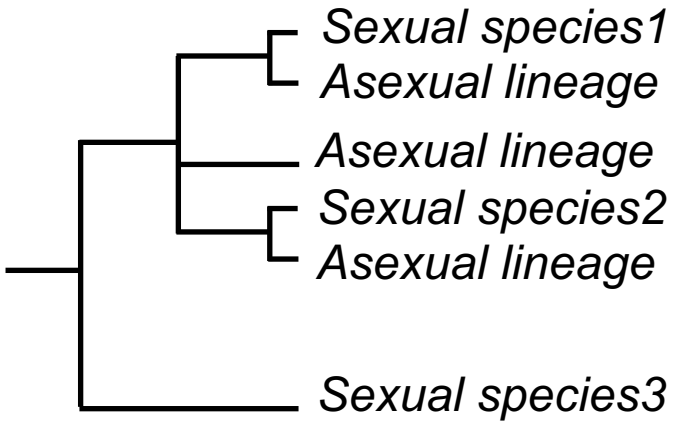
nuclear locus

Hybrid origin of asexuality

(e.g. weevils, stick insects, grasshopers)



mitochondrial locus



nuclear locus

Rate of hybridization?
Backcross possible?
Fitness of hybrids?
Polyploidization?

Better understanding of the origin and maintenance of asexual lineages

Phylogenetics
How many independent origins of asexuality?
How do we define asexual species?

Better understanding of the origin and maintenance of asexual lineages

Phylogenetics
How many independent origins of asexuality?
How do we define asexual species?

Genome evolution
Strength of selection against recombination?
Automixis (modified meiosis) or apomixis (mitosis)?
Role of polyploidy?
1 or several asexuality loci?

Better understanding of the origin and maintenance of asexual lineages

Phylogenetics
How many independent origins of asexuality?
How do we define asexual species?

Genome evolution
Strength of selection against recombination?
Automixis (modified meiosis) or apomixis (mitosis)?
Role of polyploidy?
1 or several asexuality loci?

Ecology
Geographical distribution of sexuals and asexuals?
Different ecological niches?

Brine shrimp: *Artemia parthenogenetica*

Brine shrimp: *Artemia parthenogenetica*

Brine shrimp: *Artemia parthenogenetica*

Diploids : ● *Ap2n*

Polyploids : ▲ *Ap3n*

■ *Ap4n*

⬟ *Ap5n*

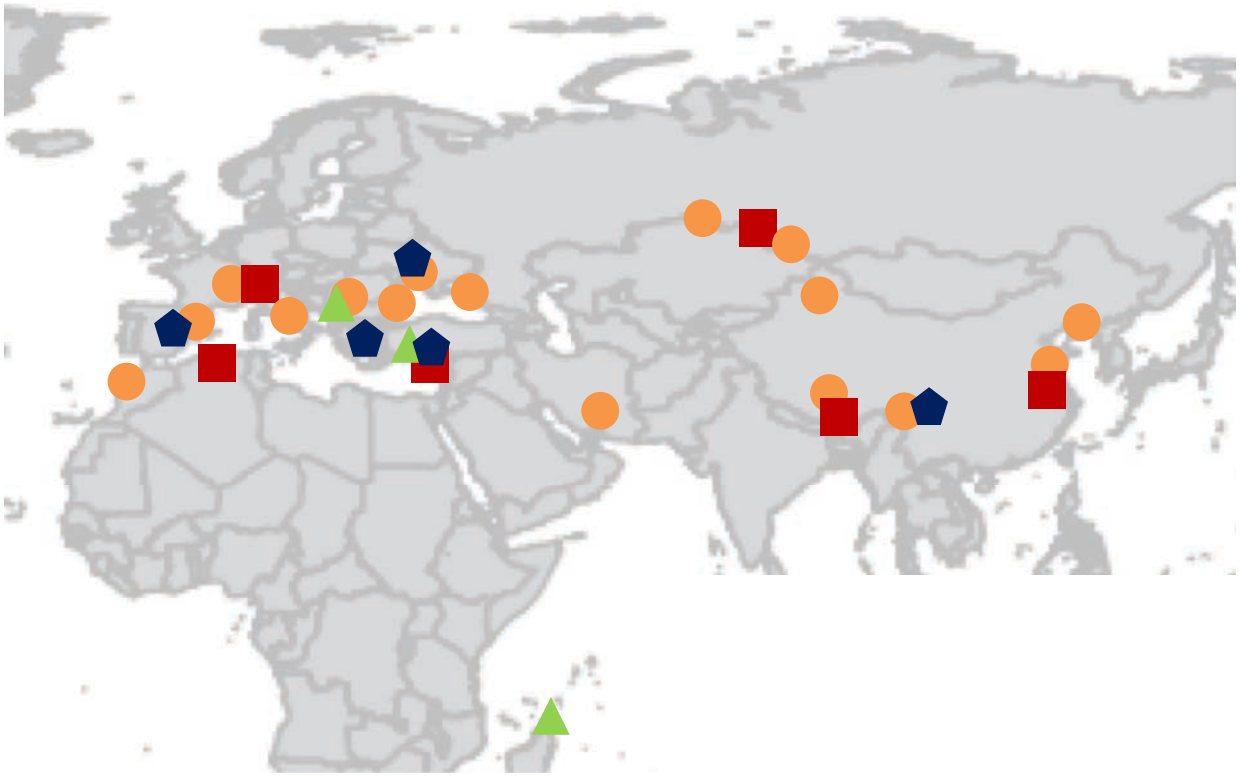
Brine shrimp: *Artemia parthenogenetica*

Diploids : ● *Ap2n* } Very high genetic diversity

Polyploids : ▲ *Ap3n*
■ *Ap4n*
⬟ *Ap5n* } Low genetic diversity

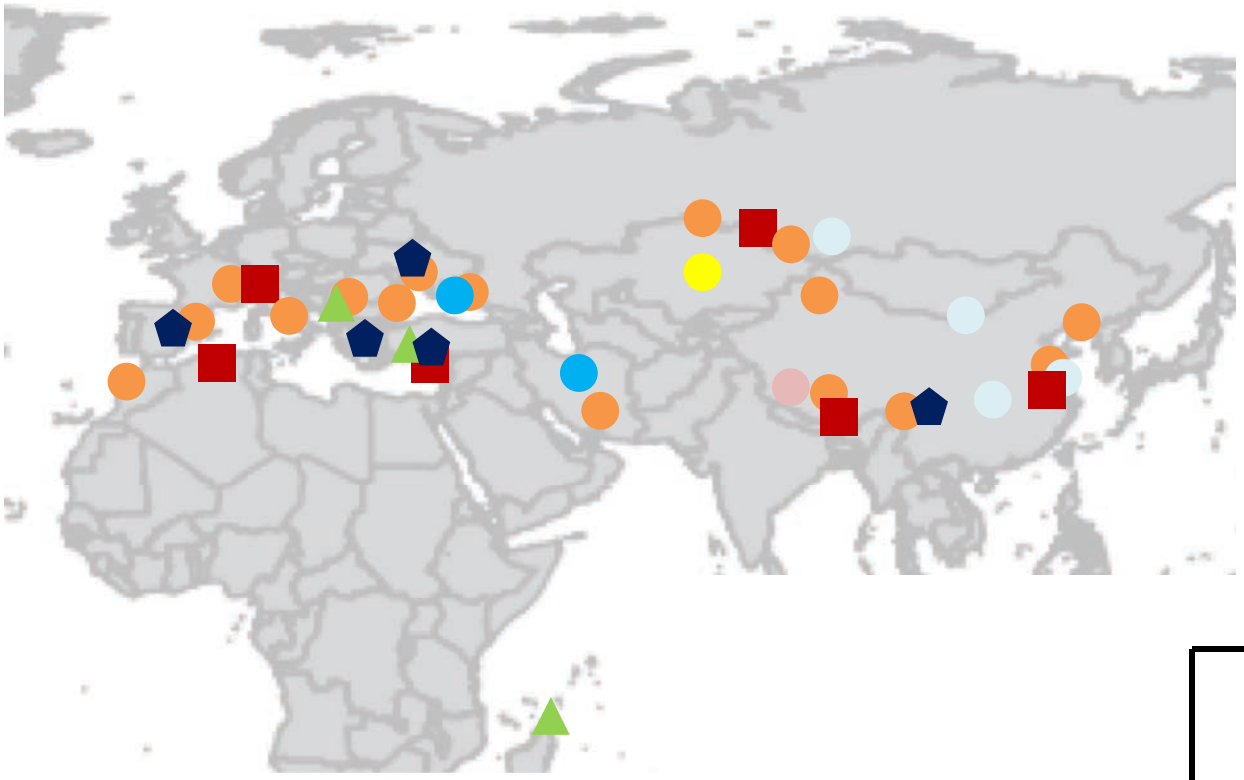
Beardmore & Abreu-Grobois 1983
Abreu-Grobois 1987

Distribution of *A. parthenogenetica* and its sexual relatives

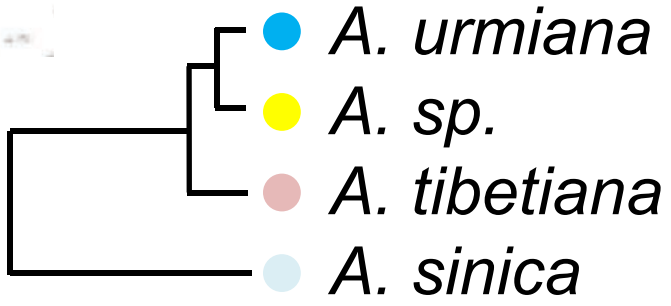


Ap2n
Ap3n
Ap4n
Ap5n

Distribution of *A. parthenogenetica* and its sexual relatives



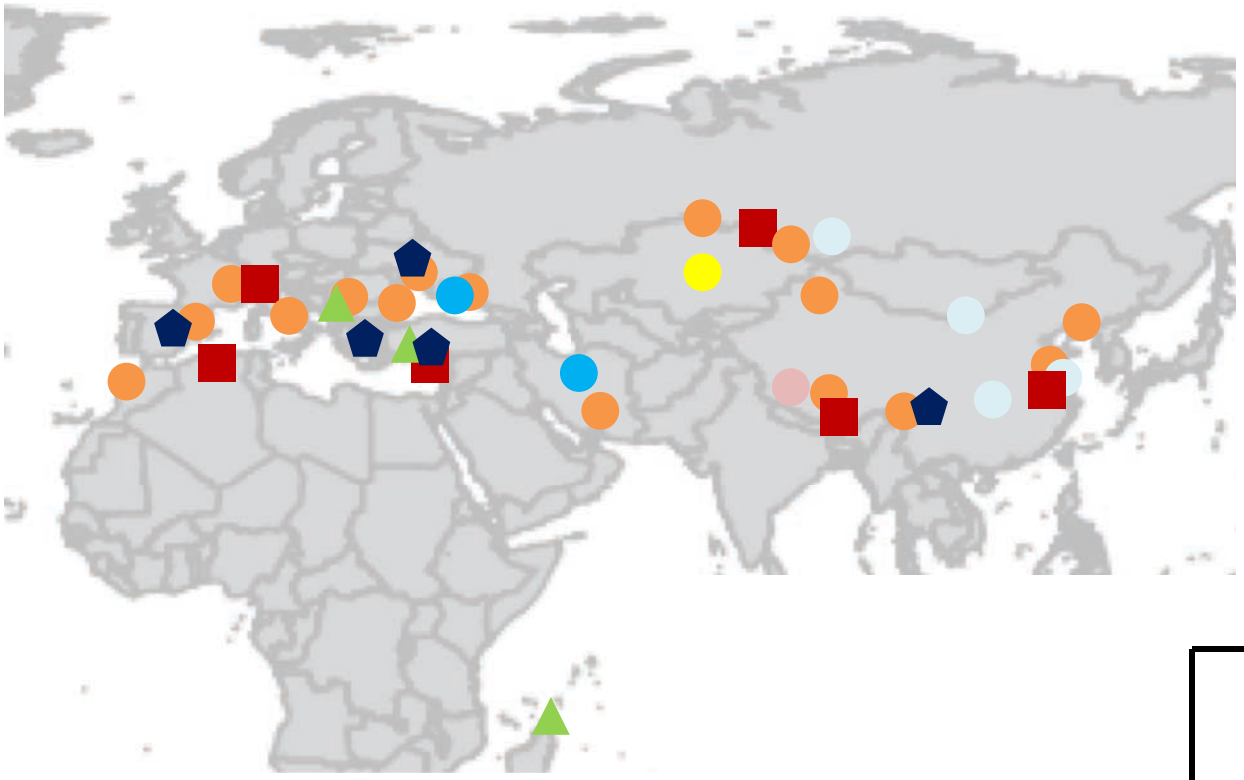
Ap2n
Ap3n
Ap4n
Ap5n



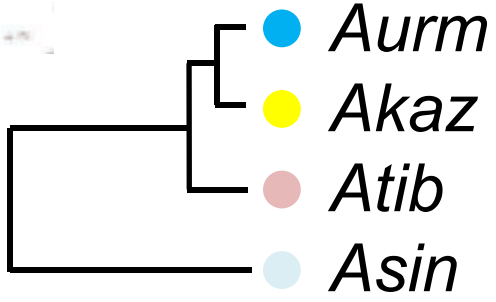
Pilla & Beardmore 1994
Baxevanis et al 2006
Eimanifar et al 2015

ITS1~2.5% => 8MY
COI~20%=> 20MY

Distribution of *A. parthenogenetica* and its sexual relatives



Ap2n
Ap3n
Ap4n
Ap5n



Pilla & Beardmore 1994
Baxevanis et al 2006
Eimanifar et al 2015

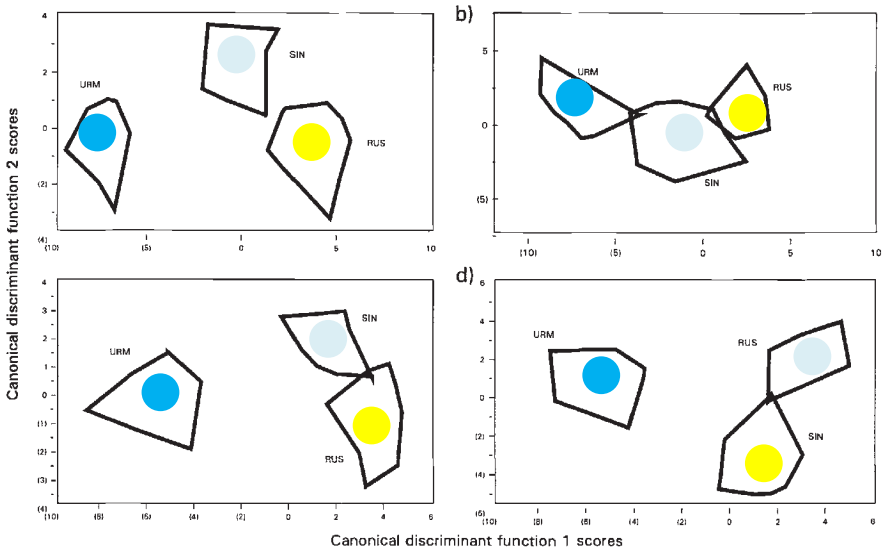
ITS1~2.5% => 8MY
COI~20%=> 20MY

Difficulty of morphological approaches

Males Females

62 g/L

82 g/L



Pilla & Beardmore 1994

Origin of asexuality in *Artemia*?

No Wolbachia infection

Maniatsi et al 2010

?

Spontaneous
Contagious
By hybridization

Origin of asexuality in *Artemia*?

No Wolbachia infection

Maniatsi et al 2010

?

Spontaneous
Contagious
By hybridization

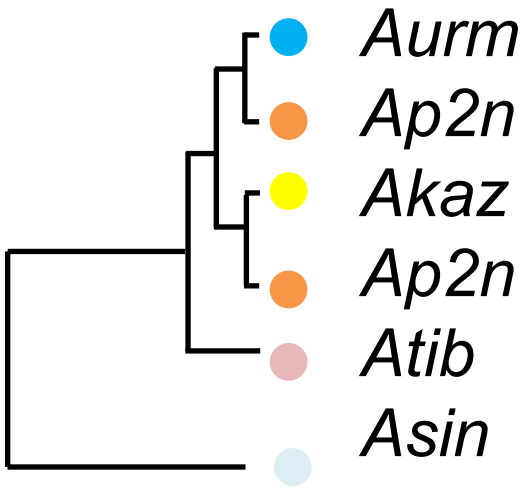
Mitochondrial data
=> 2/3 independent *Ap2n* lineages

Maniatsi et al 2011
Maccari et al 2013
Asem et al 2016

Origin of asexuality in *Artemia*?

✗ No Wolbachia infection

Maniatsi et al 2010



? Spontaneous
Contagious
By hybridization

Mitochondrial data
=> 2/3 independent *Ap2n* lineages

Maniatsi et al 2011
Maccari et al 2013
Asem et al 2016

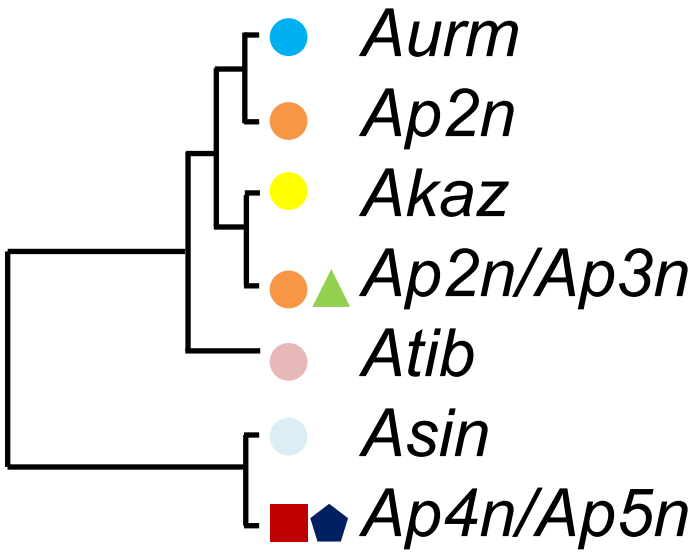
Origin of asexuality in *Artemia*?

No Wolbachia infection

Maniatsi et al 2010

?

Spontaneous
Contagious
By hybridization



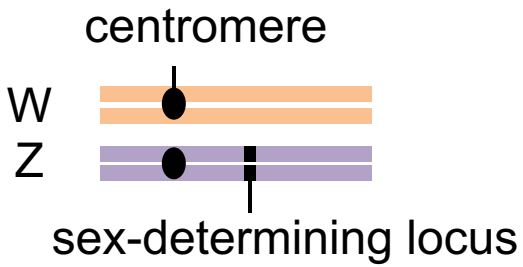
Mitochondrial data
=> 2/3 independent *Ap2n* lineages
=> Independent origin of *Ap3n vs. Ap4n* and *Ap5n*

Maniatsi et al 2011
Maccari et al 2013
Asem et al 2016

Automixis in *Ap2n*

Abonyi 1915
Artom 1931

Automixis in *Ap2n*

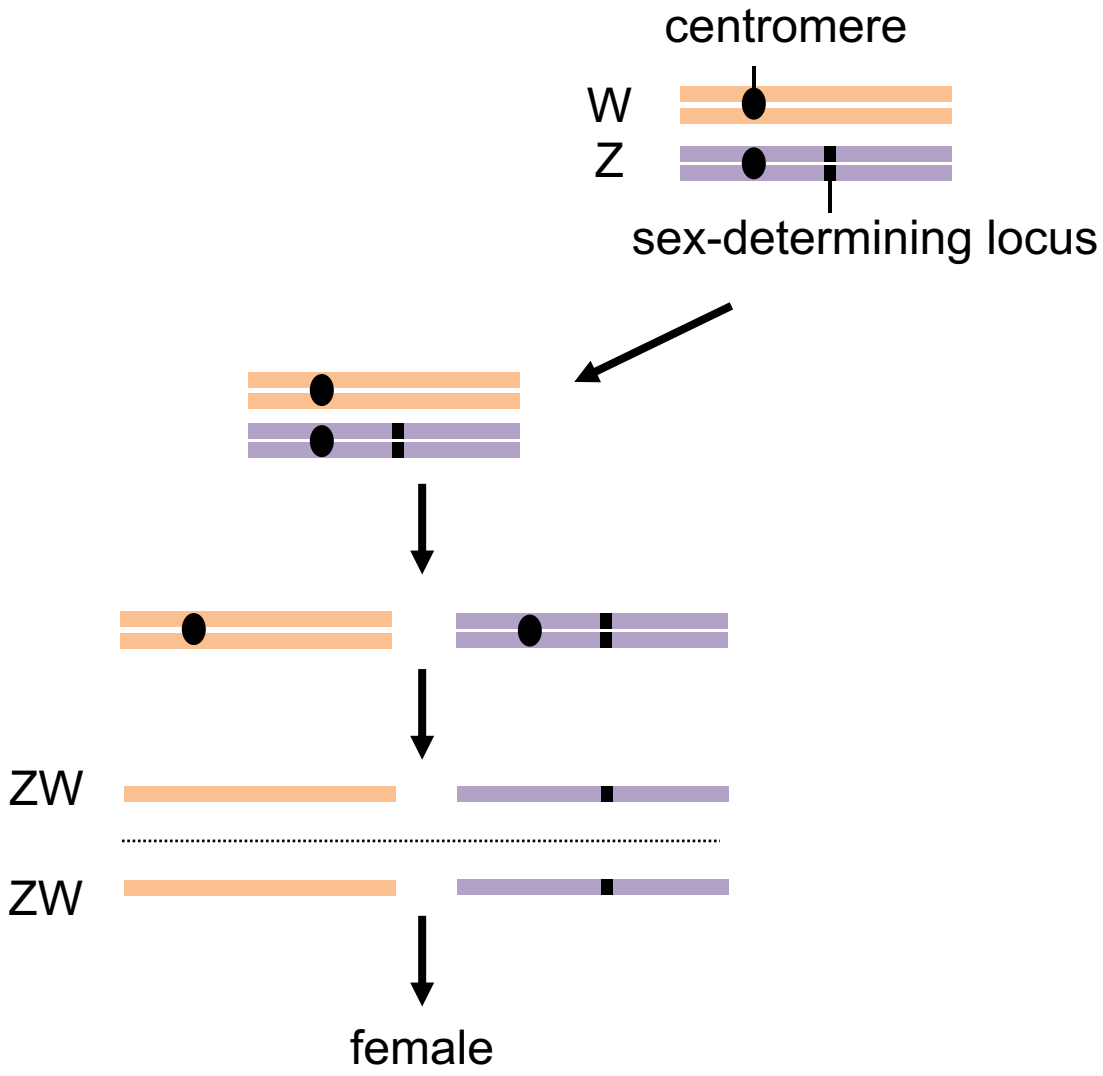


Abonyi 1915
Artom 1931

Abreu-Grobois et al 2001
Nougué et al 2015

Automixis in *Ap2n*

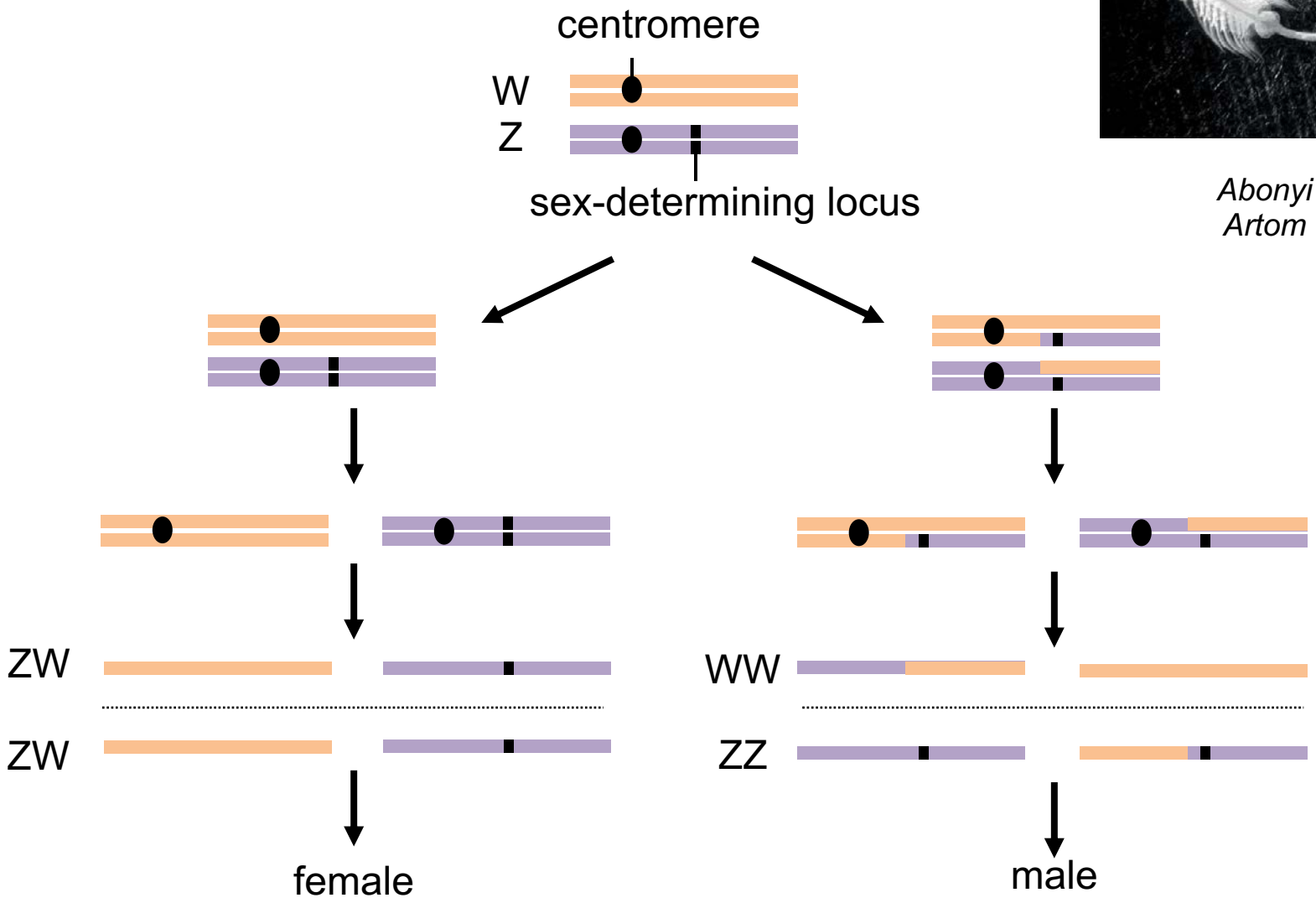
Abonyi 1915
Artom 1931



Abreu-Grobois et al 2001
Nougué et al 2015

Automixis in *Ap2n*

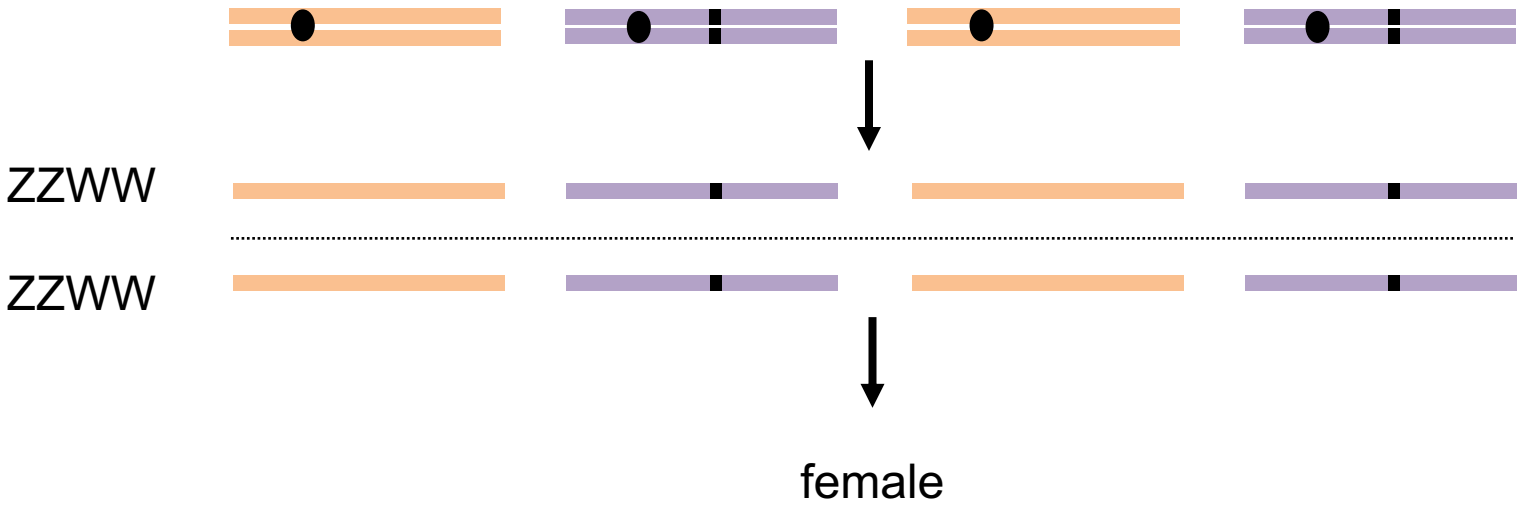
Abonyi 1915
Artom 1931



Abreu-Grobois et al 2001
Nougué et al 2015

Apomixis in *Ap3n*, *Ap4n*, *Ap5n*

Brauer 1893
Artom 1906
Barigozzi 1964



Ap3n and *Ap5n* with automixis as in *Ap2n*

Goldschmidt 1952

Rare males never observed
(e.g. in cultures of ~200000 *Ap3n, Ap4n* and *Ap5n*)

Goldschmidt 1952
Chang et al 2017

Contagious asexuality through rare males?

Important variation among *Ap2n* lineages (0-1.7%)

MacDonald & Browne 1987
Maccari et al 2013
Chang et al 2017

↓

Contagious asexuality through rare males?

Important variation among *Ap2n* lineages (0-1.7%)

MacDonald & Browne 1987
Maccari et al 2013
Chang et al 2017

Crosses between rare *Ap2n* males and *Aurm* or *Akaz* females

↓

Some female F1/F2 produce offspring asexually

Maccari et al 2013
Boyer, unpublished

Contagious asexuality through rare males?

Important variation among *Ap2n* lineages (0-1.7%)

MacDonald & Browne 1987
Maccari et al 2013
Chang et al 2017

Crosses between rare *Ap2n* males and *Aurm* or *Akaz* females

↓

Some female F1/F2 produce offspring asexually

Maccari et al 2013
Boyer, unpublished

Occurence of contagious asexuality in natural *Ap2n* populations?
Recent origin of *Ap2n* lineages with high proportions of rare males?
Rare males involved in the origin of polyploids?

Outline

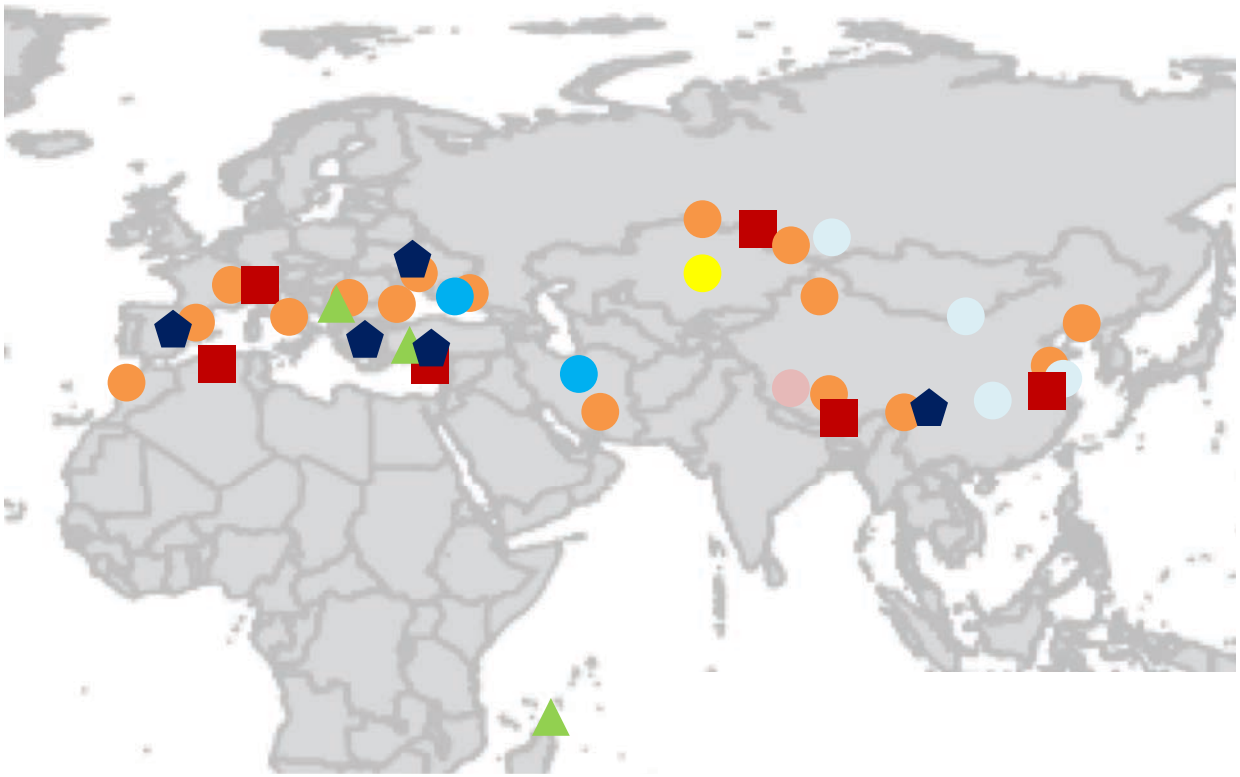
1. Number of maternal origins and diversity levels in *Ap2n-5n*

   - Number of mitochondrial haplotypes
   - Genome size
   - Levels of nuclear diversity

2. Mode(s) of origin of *Ap2n*
   - Monophyly of clades based on mitochondrial haplotypes?
   - Comparison of different evolutionary scenarios

3. Mode(s) of origin of *Ap3n*, *Ap4n*, *Ap5n*

Methods



Ap2n
Ap3n
Ap4n
Ap5n
Aurm
Akaz
Atib
Asin

Samples from 37 populations
206 individuals: genome size (flow cytometry)
365 individuals: mitochondrial haplotype (COI)
489 individuals: multilocus genotype (12 microsatellites)

Outline

1. Number of maternal origins and diversity levels in *Ap2n-5n*

   - Number of mitochondrial haplotypes
   - Genome size
   - Levels of nuclear diversity

2. Mode(s) of origin of *Ap2n*
   - Monophyly of clades based on mitochondrial haplotypes?
   - Comparison of different evolutionary scenarios

3. Mode(s) of origin of *Ap3n*, *Ap4n*, *Ap5n*

Mitochondrial haplotype

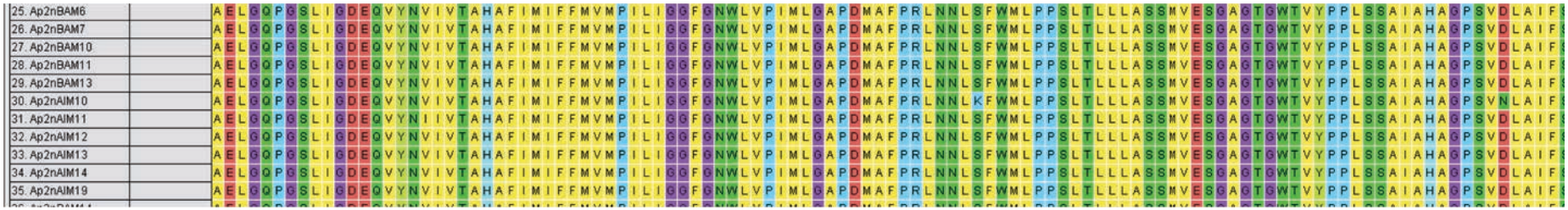
365 mitochondrial haplotype
~1000 sequences from NCBI

Mitochondrial haplotype

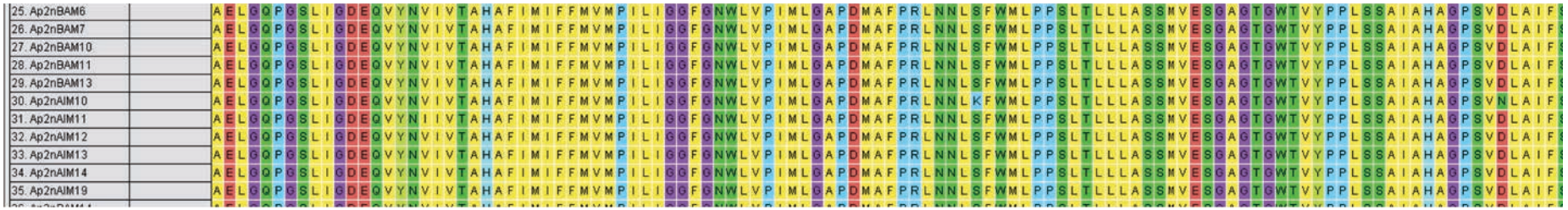
365 mitochondrial haplotype
~1000 sequences from NCBI

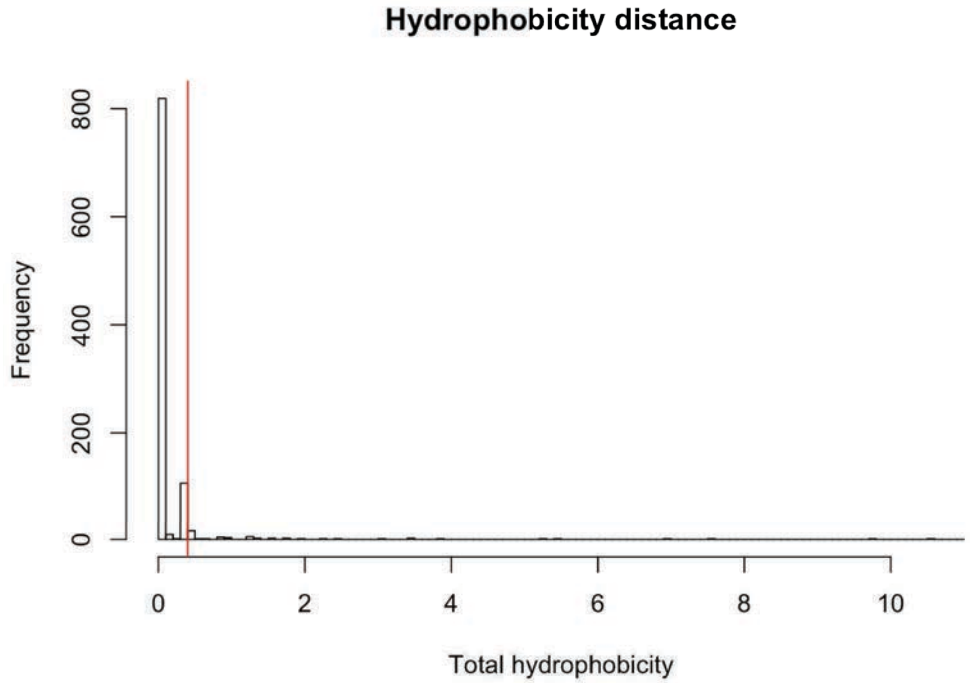
```
Contig-CIT-B1-col1          TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-B5-col1          TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-bte2-col3        TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-bte4-col2        TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-bte4-col3        TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-bte_5-col2       TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-B3-col1          TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-B4-col1          TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
Contig-CIT-B2-col2          TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
>ContigCIT4_                TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATC
>ContigCIT3_                TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATY
>ContigCIT1                 TAAACTTTATTACTACTATTATTAATATRCGRCCTCAGTCAATATCTATC
>ContigCIT2                 TAAACTTTATTACTACTATTATTAATATGCGGCCTCAGTCAATATCTATY
>Contig-BUJ5                TAAACTTCATTACCACTATTATCAATATACGACCTCAGTCAATATCTATT
                            ******* ***** ******** ***** ** ******************
```

Numt trimming

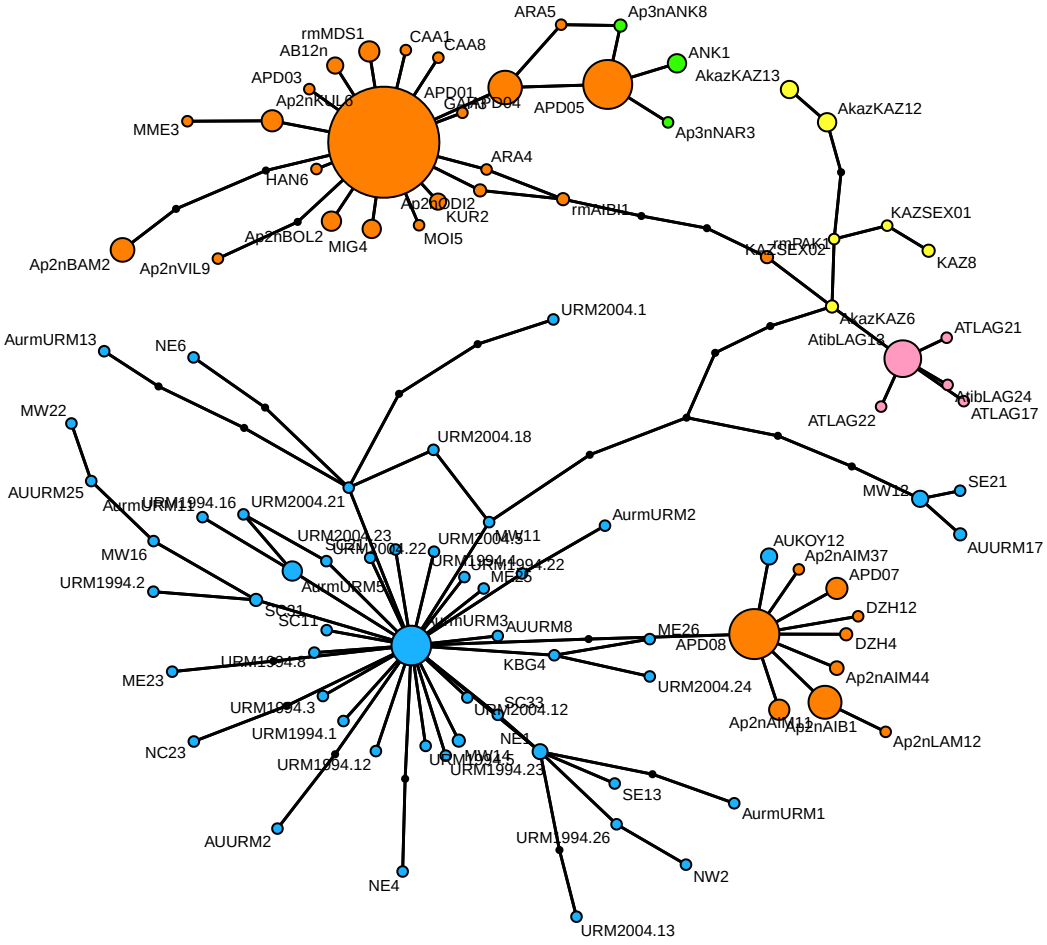


Numt trimming

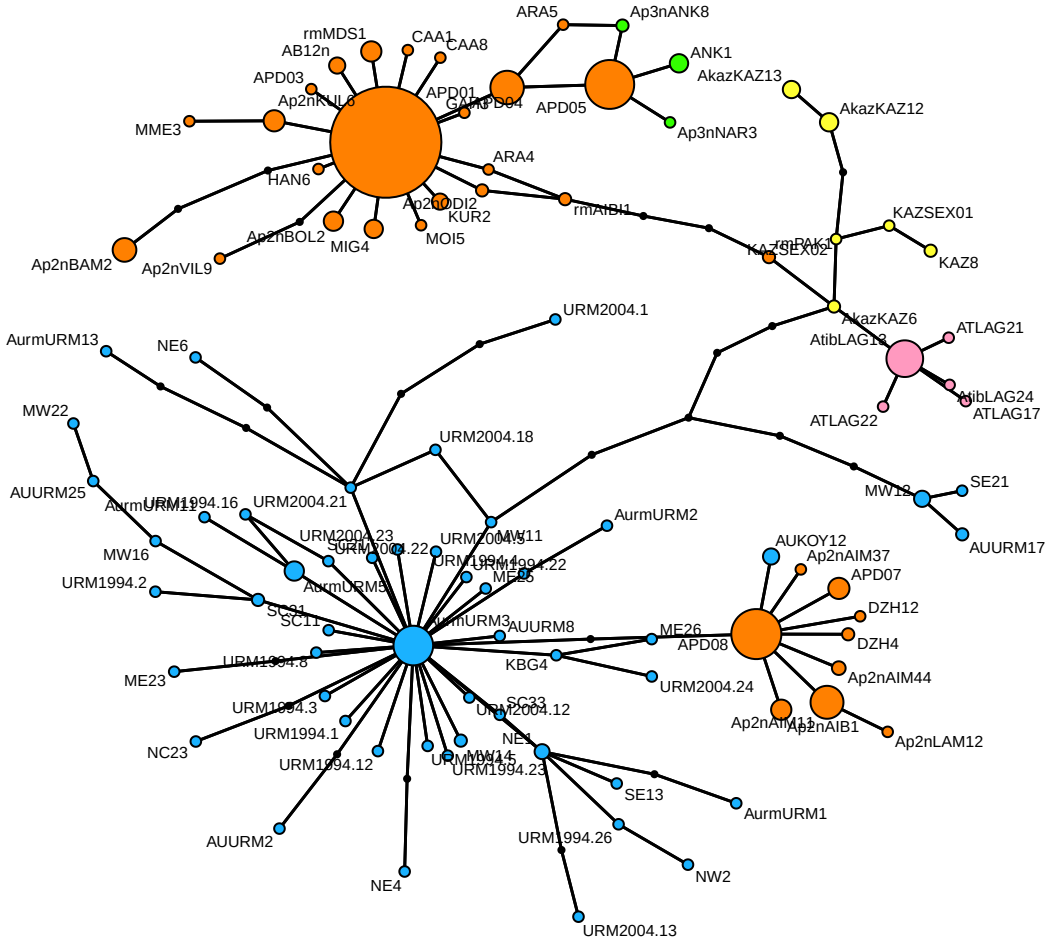


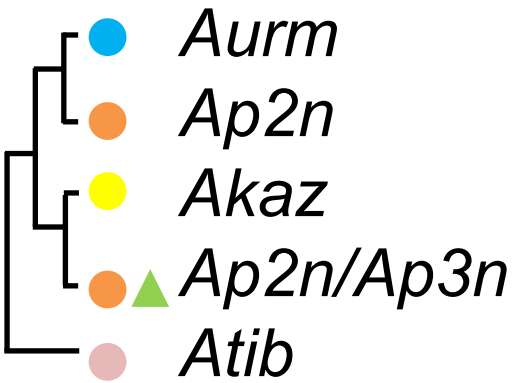


Mitochondrial haplotype of *Ap2n* and *Ap3n*



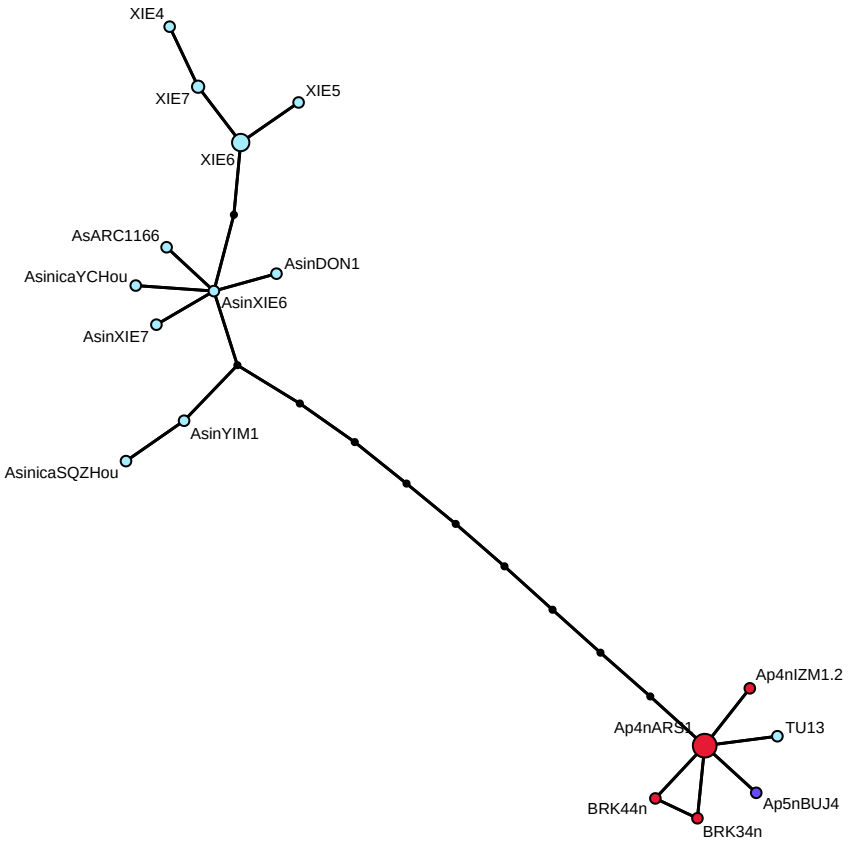
Mitochondrial haplotype of *Ap2n* and *Ap3n*

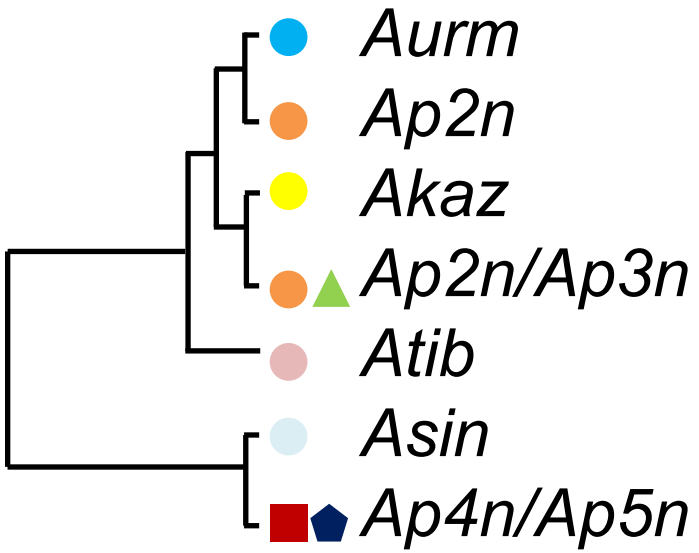




Maniatsi et al 2011
Maccari et al 2013
Asem et al 2016

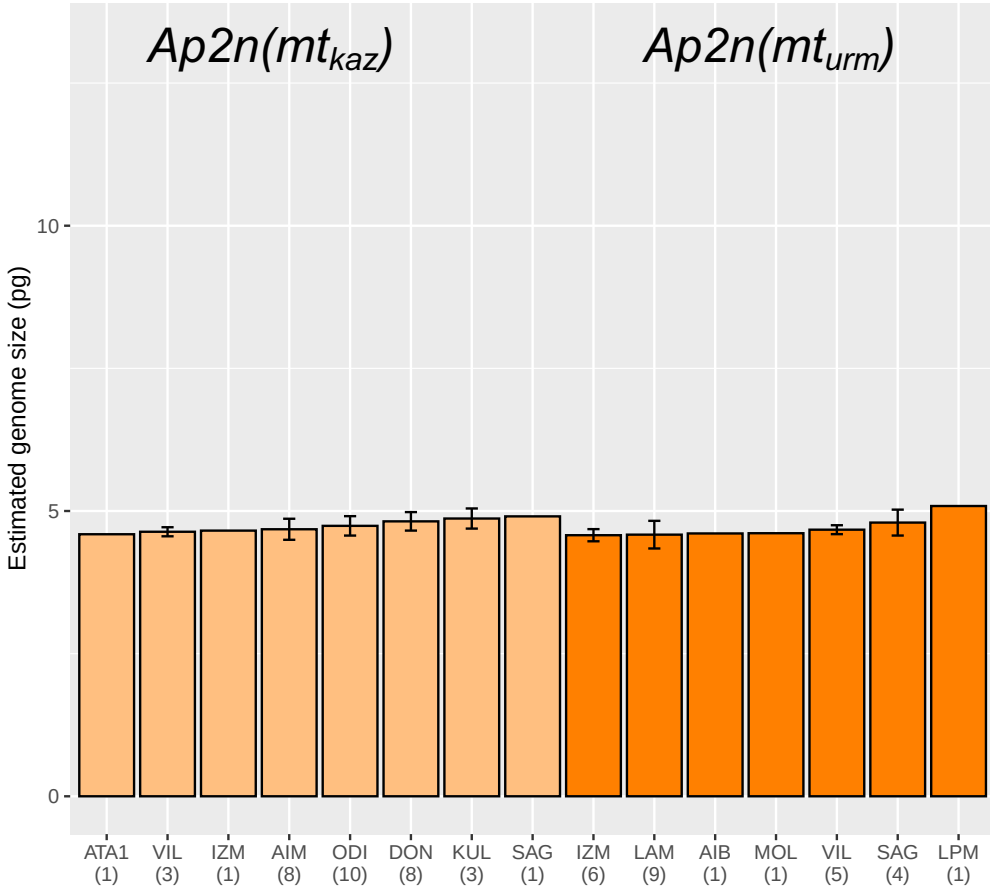
Mitochondrial haplotype of *Ap4n* and *Ap5n*

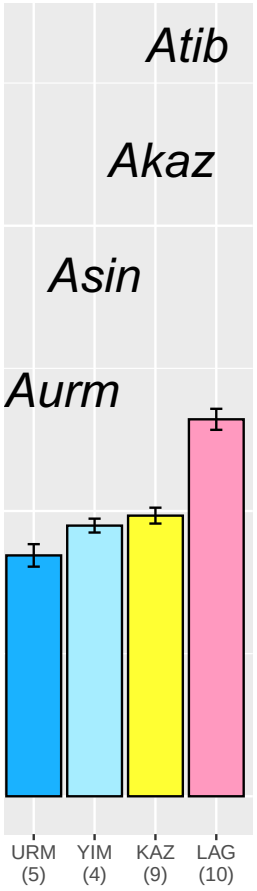




Maniatsi et al 2011
Maccari et al 2013
Asem et al 2016

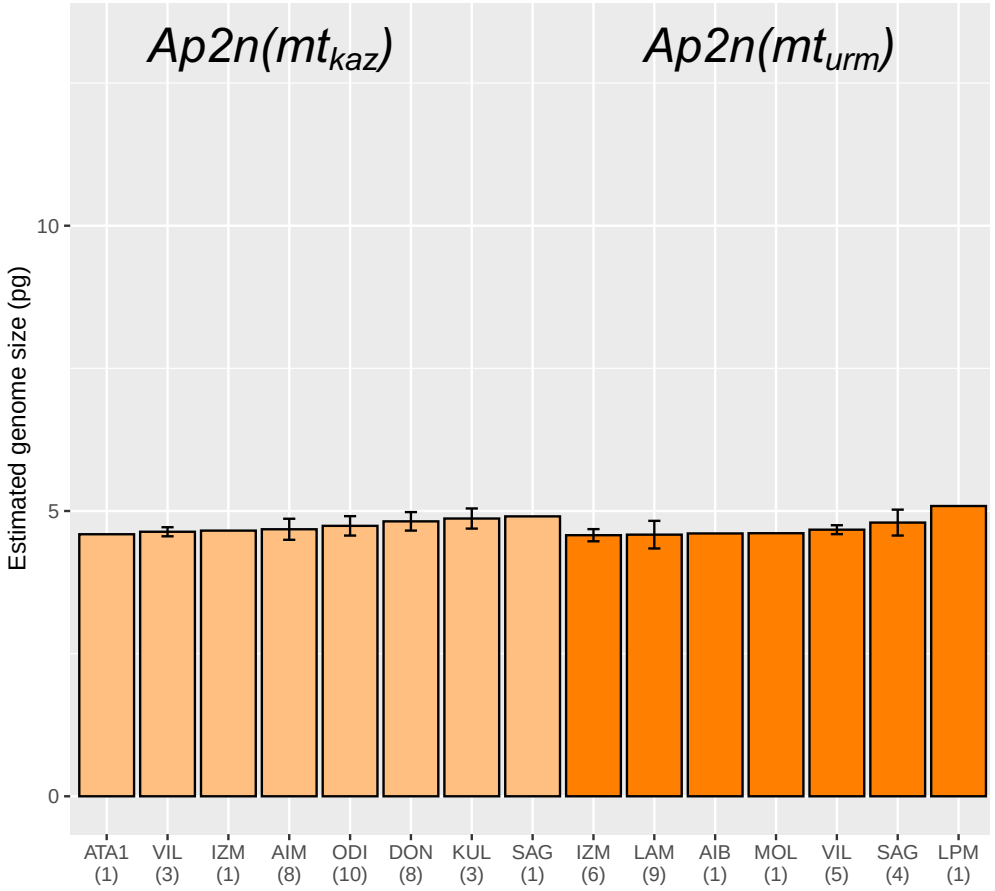
Genome size estimates

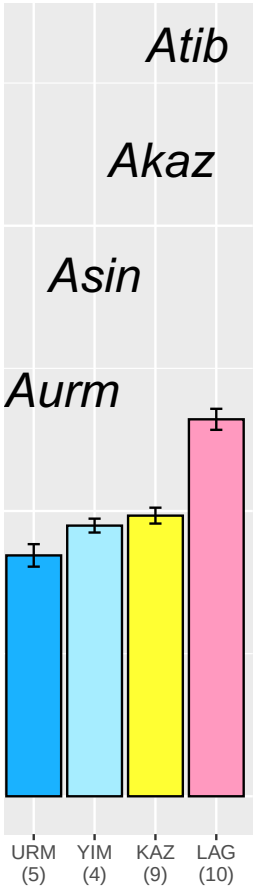




Not difference between *Ap2n(mt$_{kaz}$)* and *Ap2n(mt$_{urm}$)*
Ap2n significantly higher than *Aurm* and lower than *Akaz*

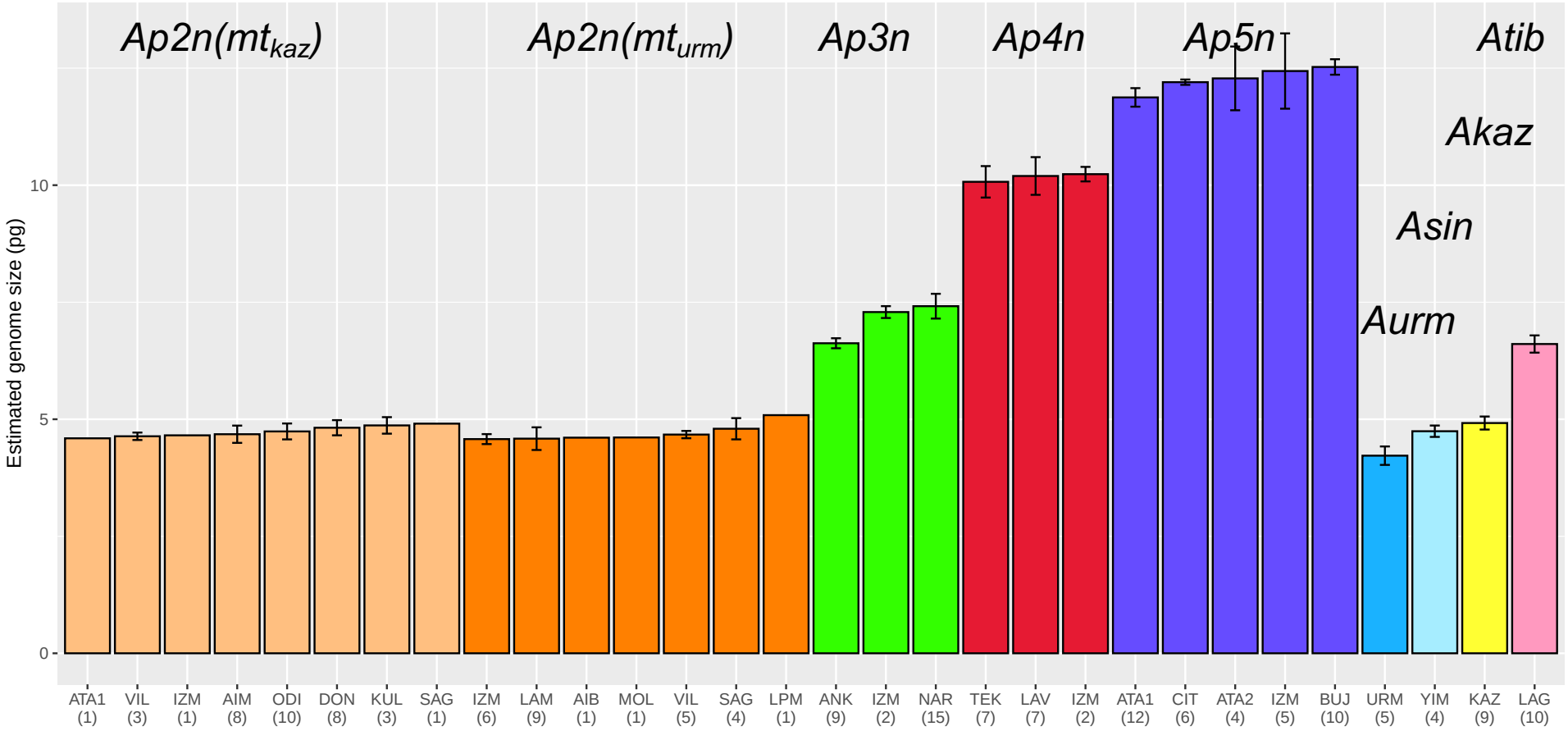
Genome size estimates





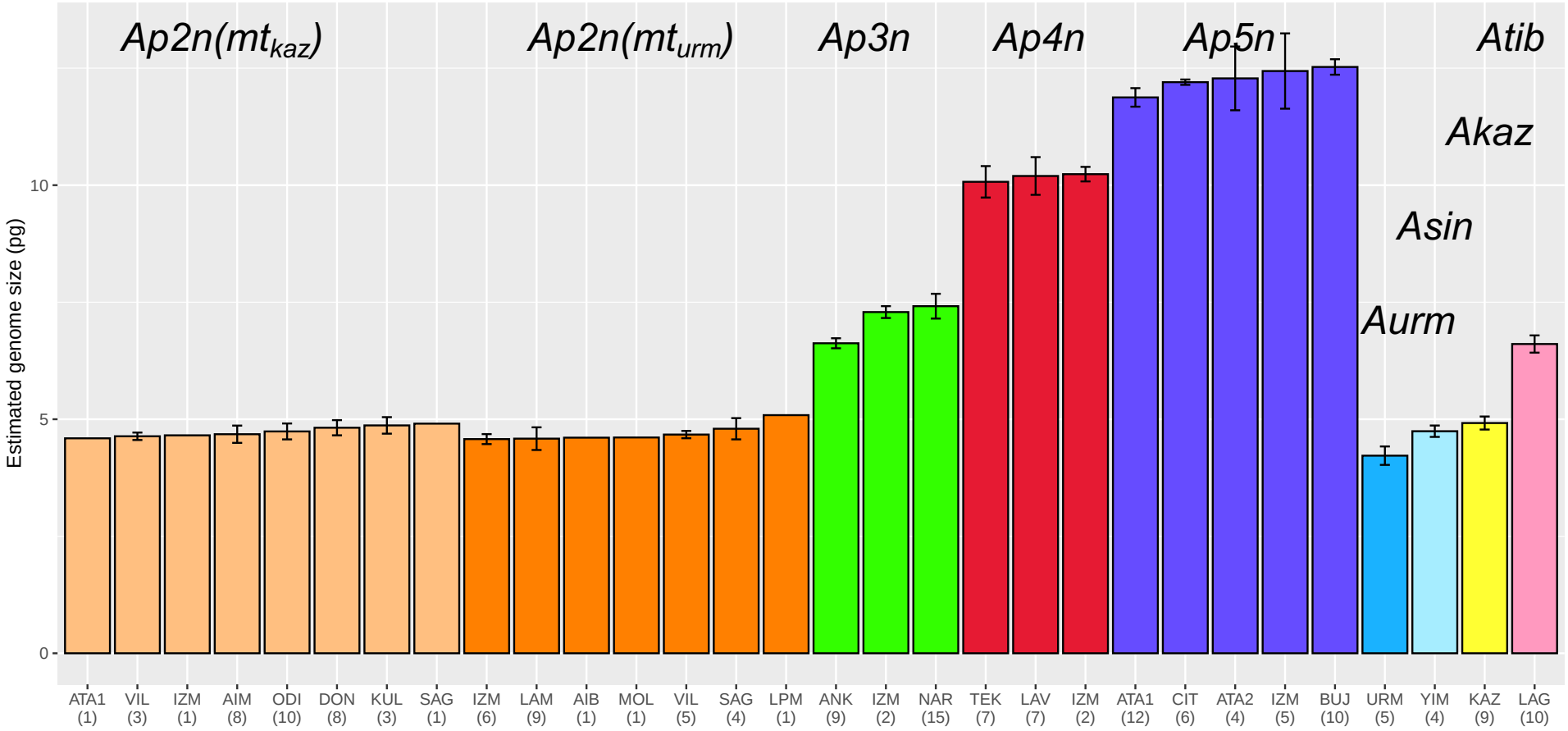
Not difference between *Ap2n(mt$_{kaz}$)* and *Ap2n(mt$_{urm}$)*
Ap2n significantly higher than *Aurm* and lower than *Akaz*
Ap4n significantly higher than twice *Asin*

Genome size estimates



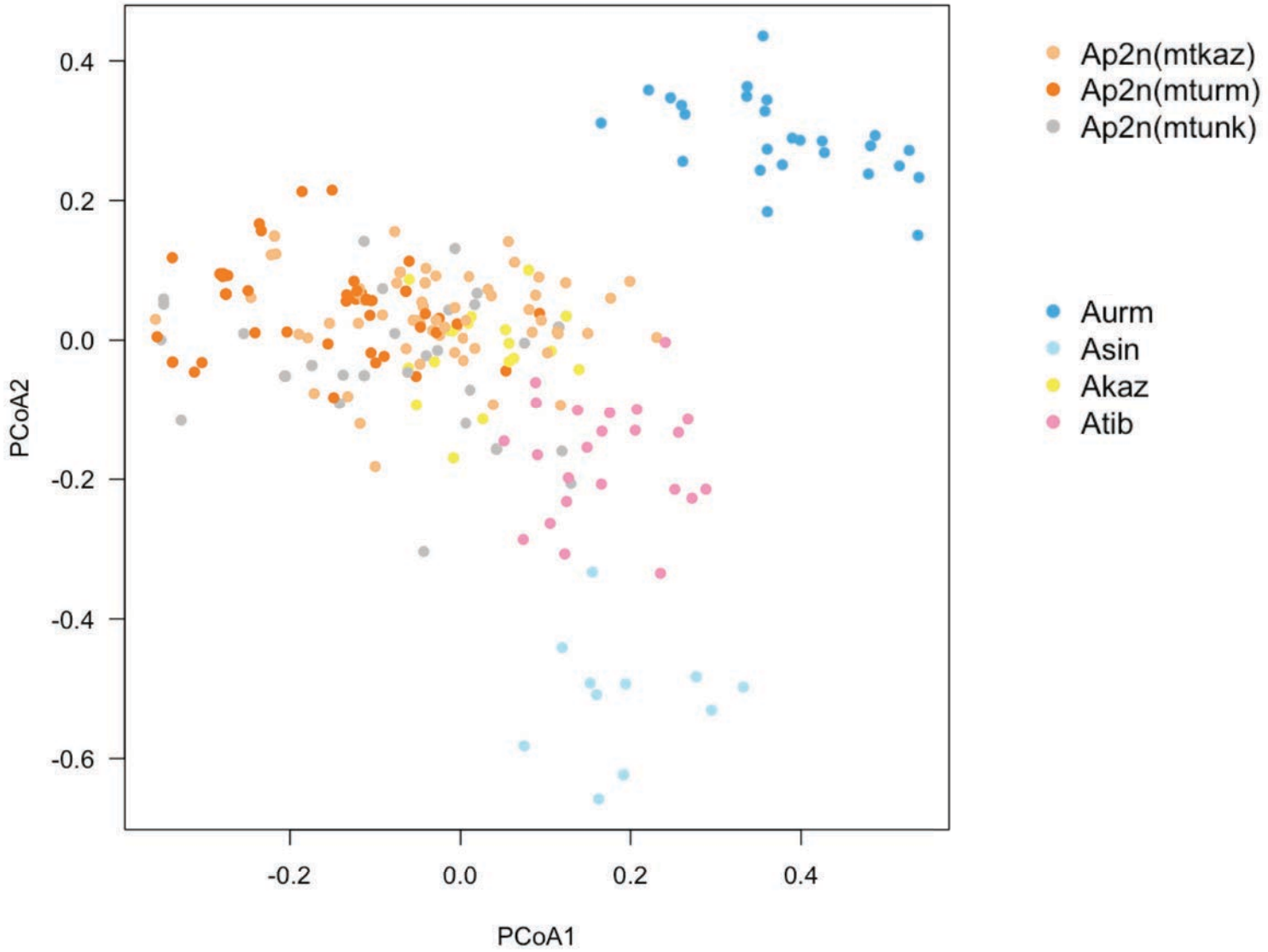
Not difference between *Ap2n(mt$_{kaz}$)* and *Ap2n(mt$_{urm}$)*
Ap2n significantly higher than *Aurm* and lower than *Akaz*
Ap4n significantly higher than twice *Asin*

Genome size estimates

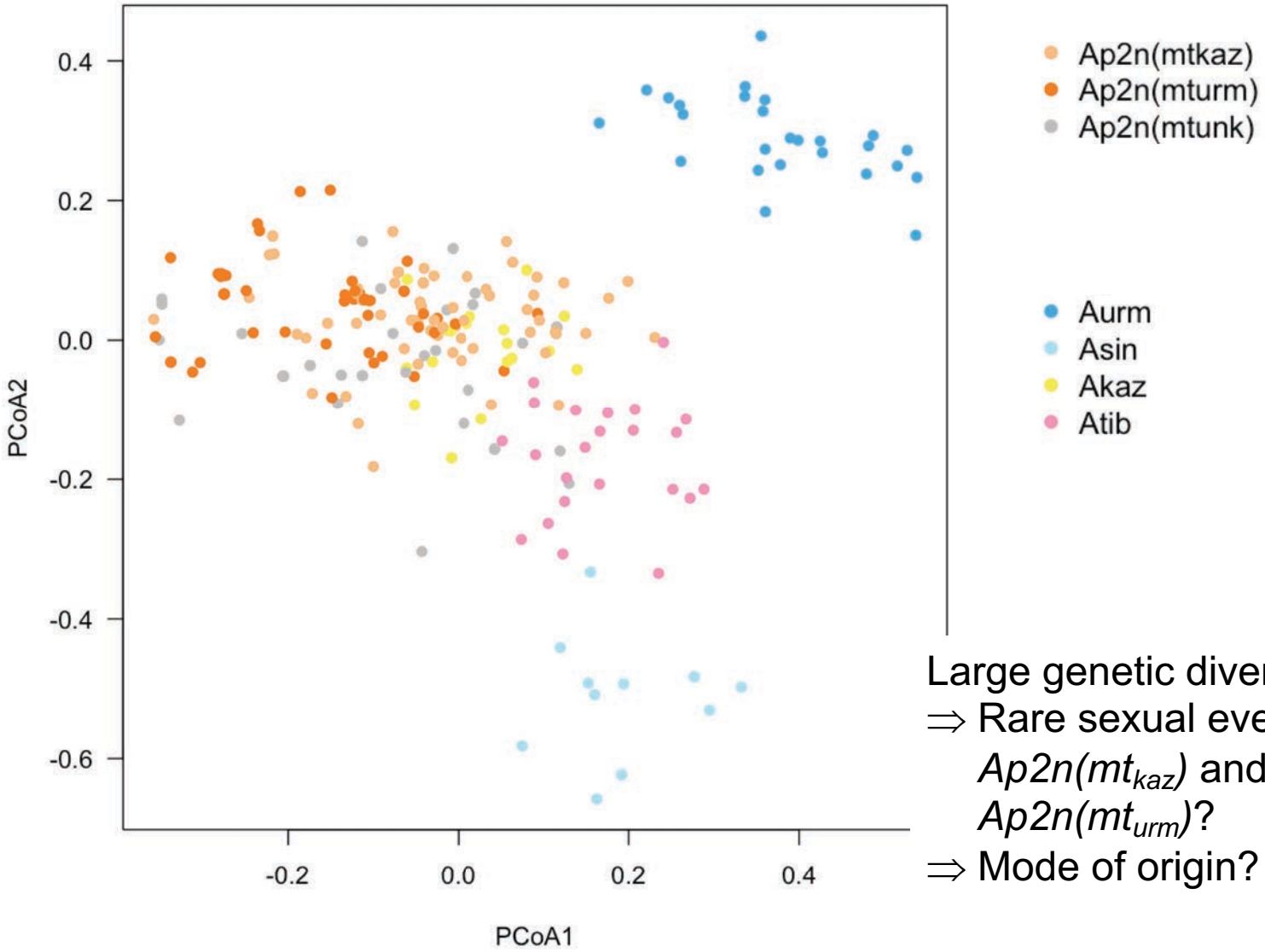


Not difference between *Ap2n(mt$_{kaz}$)* and *Ap2n(mt$_{urm}$)*
Ap2n significantly higher than *Aurm* and lower than *Akaz*
Ap4n significantly higher than twice *Asin*
➔Spontaneous origin not likely
➔Contagious or hybrid origin more likely

Genetic distance of *Ap2n*



Genetic distance of *Ap2n*

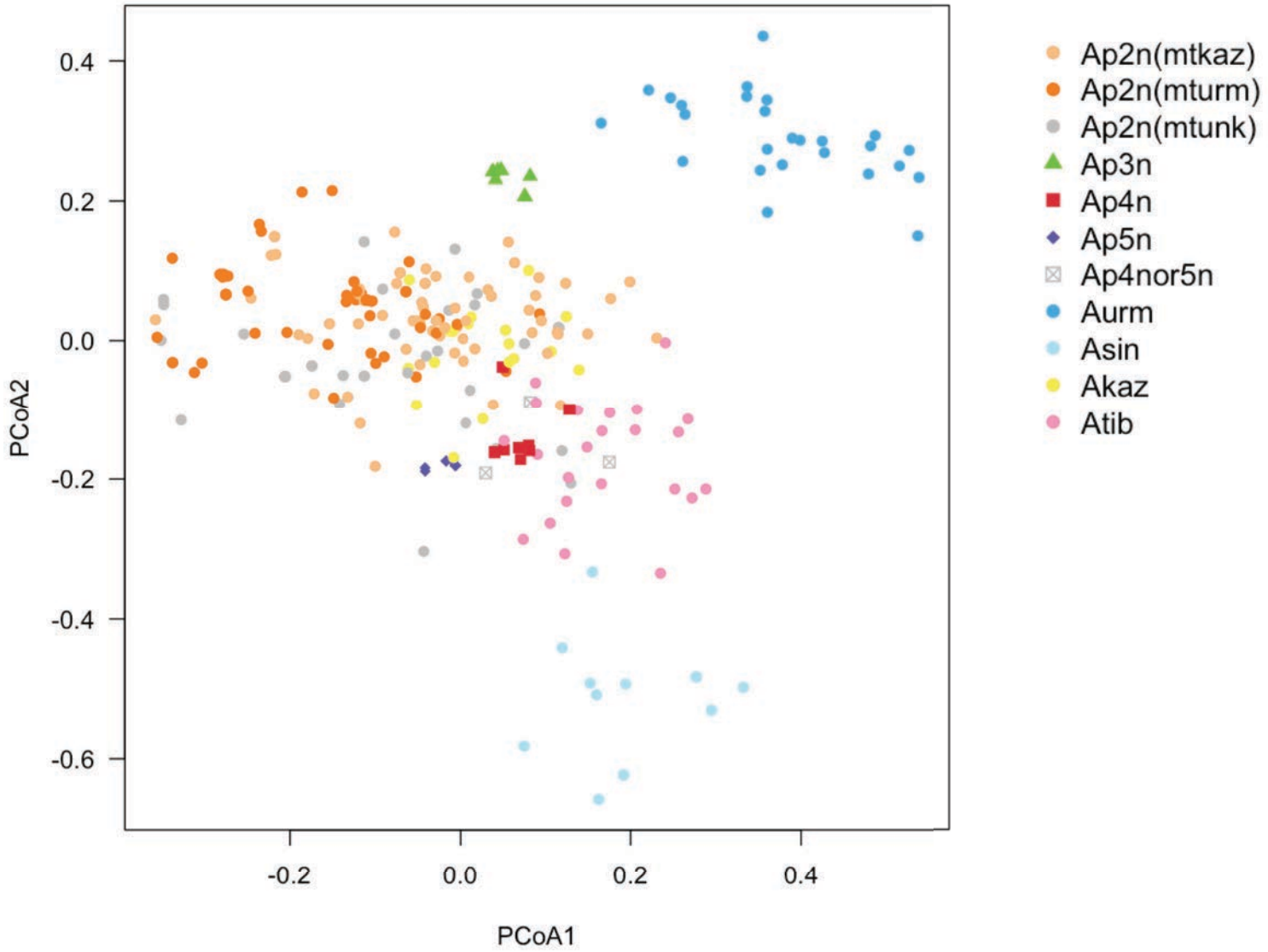


Large genetic diversity of *Ap2n*

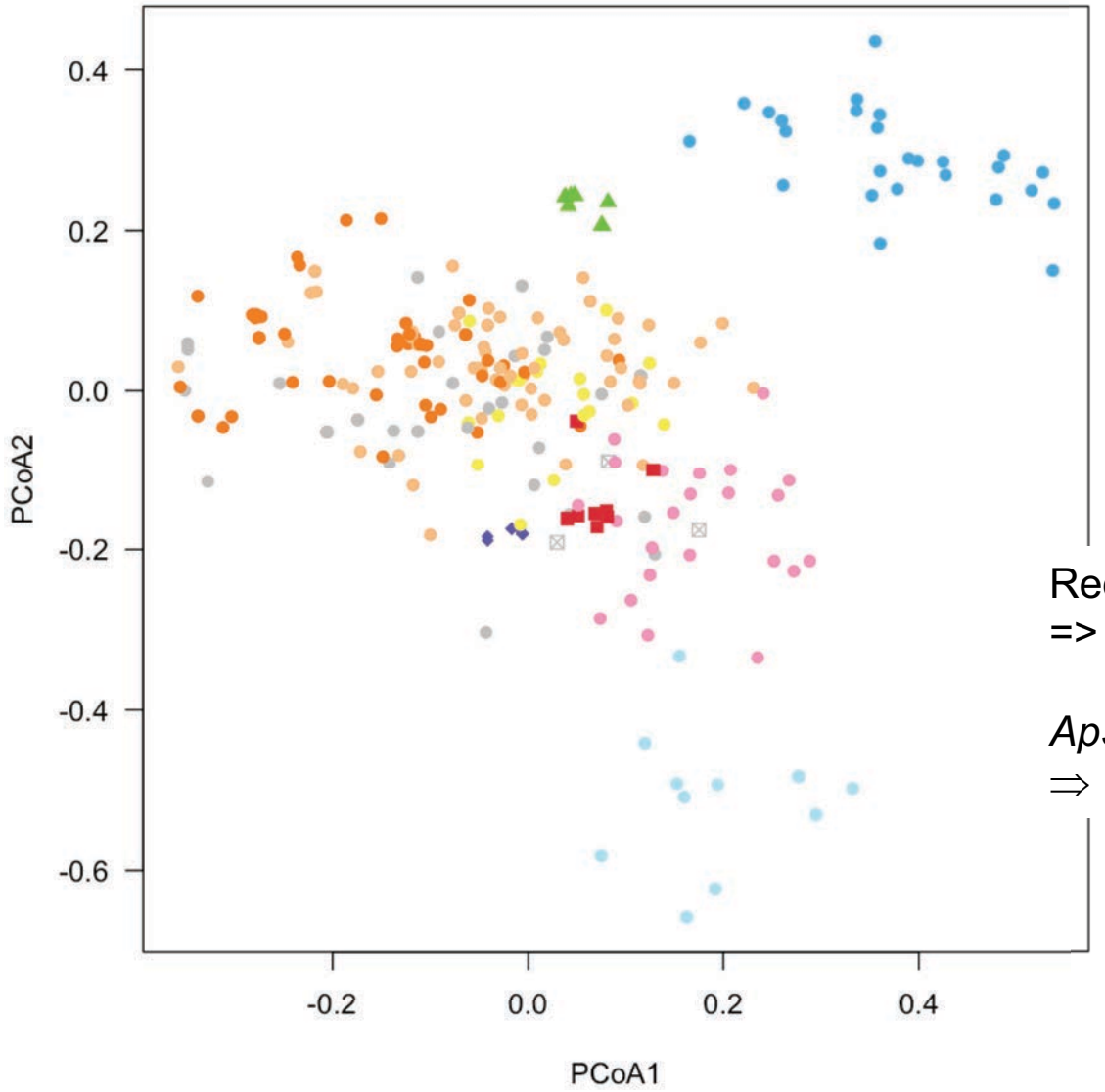
⇒ Rare sexual events between *Ap2n*(mt_{kaz}) and *Ap2n*(mt_{urm})?

⇒ Mode of origin?

Genetic distance of *Ap3n*, *Ap4n*, *Ap5n*



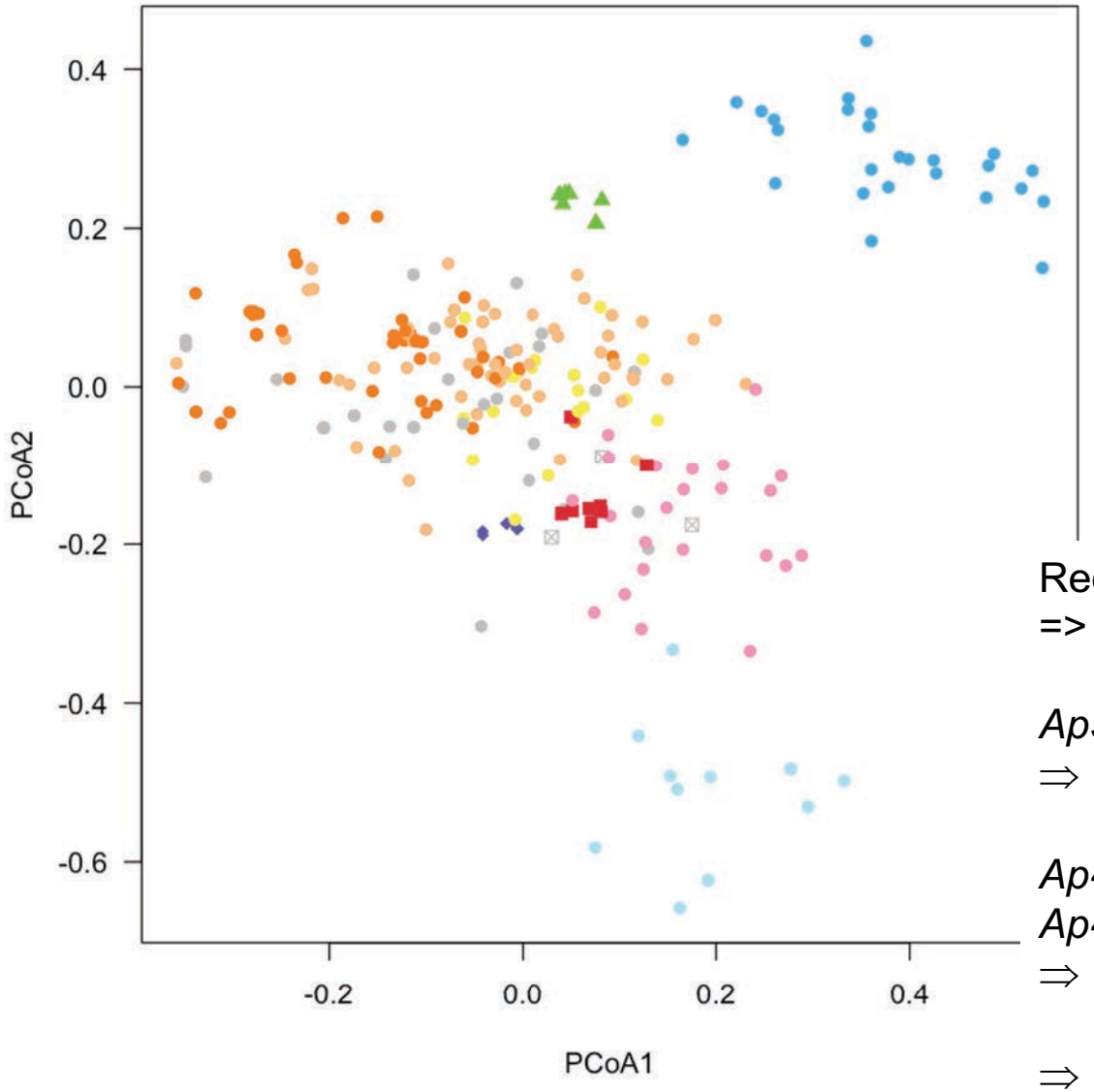
Genetic distance of *Ap3n*, *Ap4n*, *Ap5n*



Reduced genetic diversity of polyploids
=> single origin

Ap3n between *Ap2n(mt$_{kaz}$)* & *Aurm*
$\Rightarrow$ hybrid origin?

Genetic distance of *Ap3n*, *Ap4n*, *Ap5n*



Reduced genetic diversity of polyploids
=> single origin

Ap3n between *Ap2n(mt$_{kaz}$)* & *Aurm*
⇒ hybrid origin?

Ap4n between *Asin* & *Akaz*/*Ap2n*
Ap4n with two alleles
⇒ hybrid origin followed by endoduplication?
⇒ *Ap5n* derived from *Ap4n*

Outline

1. Number of maternal origins and diversity levels in *Ap2n-5n*

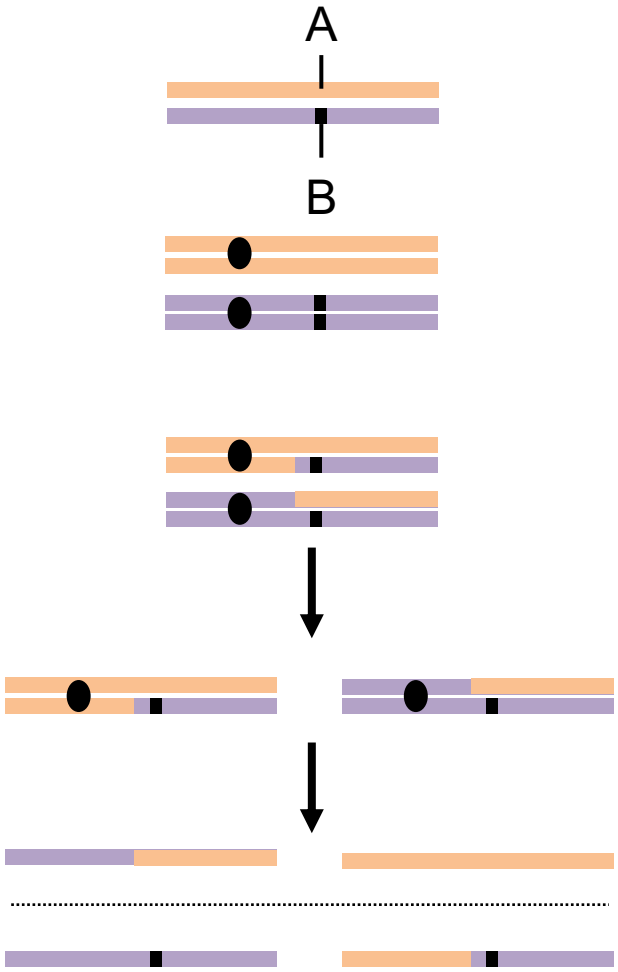
   - Number of mitochondrial haplotypes
   - Genome size
   - Levels of nuclear diversity

2. Mode(s) of origin of *Ap2n*
   - Monophyly of clades based on mitochondrial haplotypes?
   - Comparison of different evolutionary scenarios

3. Mode(s) of origin of *Ap3n*, *Ap4n*, *Ap5n*

Automixis in *Ap2n*

Female AB



Female AA

Female BB

Nougué et al 2015

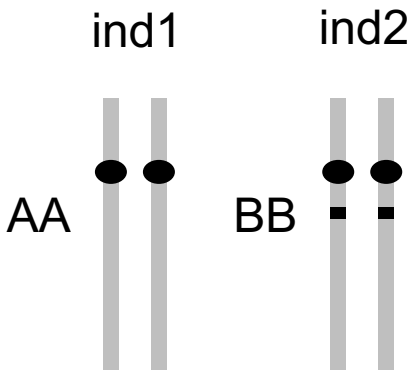
Effect of both mutation (μ) and recombination (r)

New genetic distance for automictic species

Loci close to centromere

Fis≃ -1

μ >> r



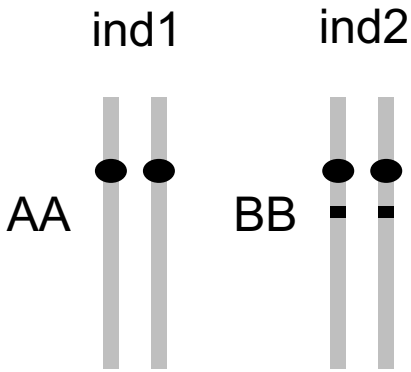
distance ind1/ind2=2

New genetic distance for automictic species

Loci close to centromere

Fis≃ -1

μ >> r

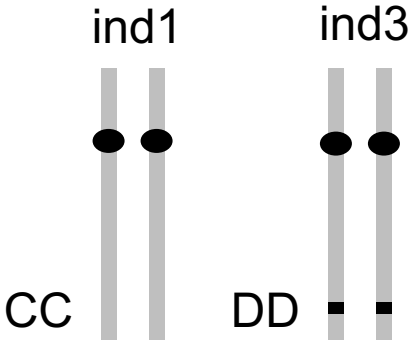


distance ind1/ind2=2

Loci far away from centromere

Fis≃ +1

μ << r

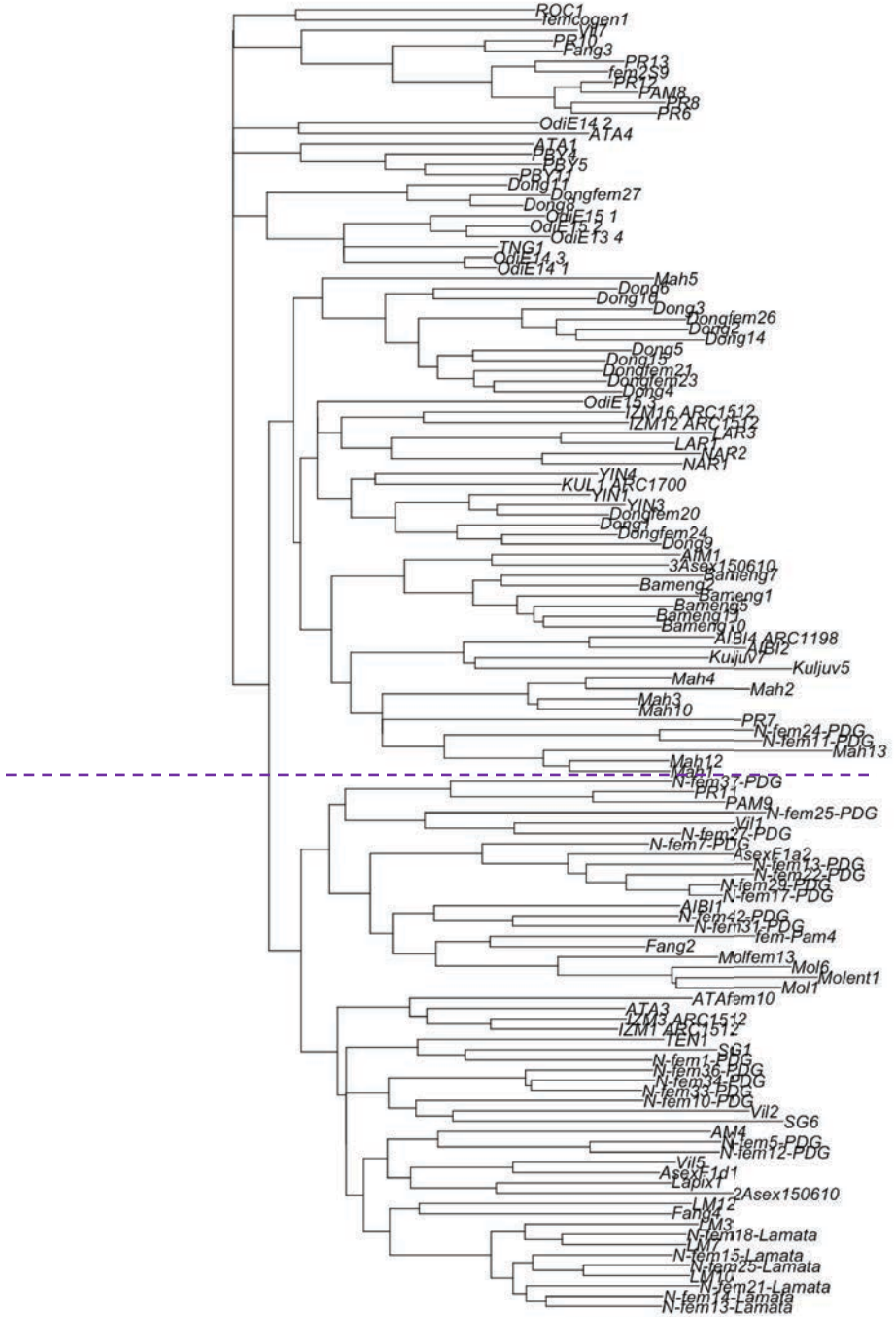


distance ind1/ind3=10^{-3}

Application to *Ap2n*

Distance matrix between 127 individuals

NJ tree



Application to *Ap2n*

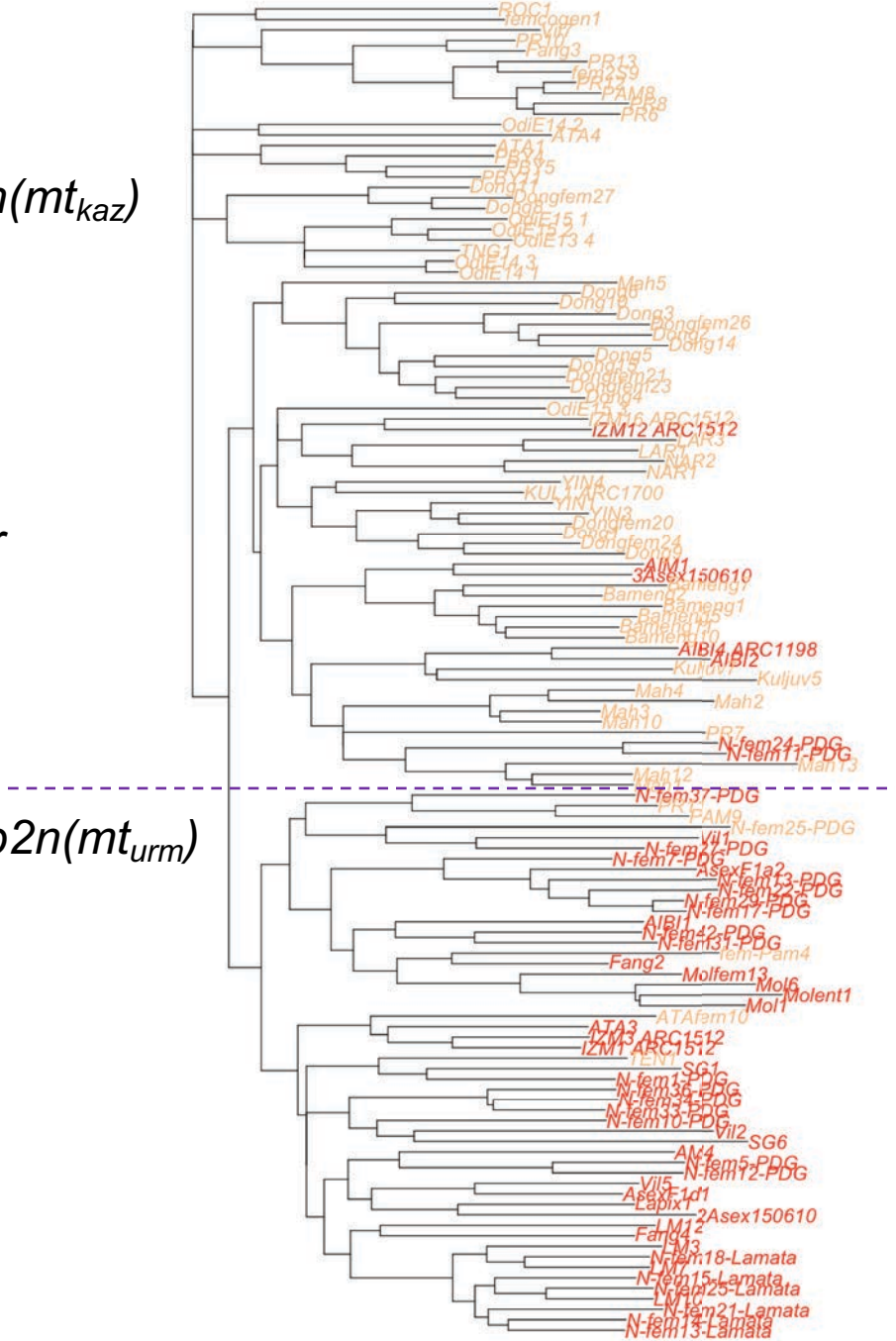
Ap2n(mt_{kaz})

Distance matrix between 127 individuals

NJ tree

➔ Significant association between nuclear genetic distance and mitochondrial haplotype (P<0.001)

Ap2n(mt_{urm})



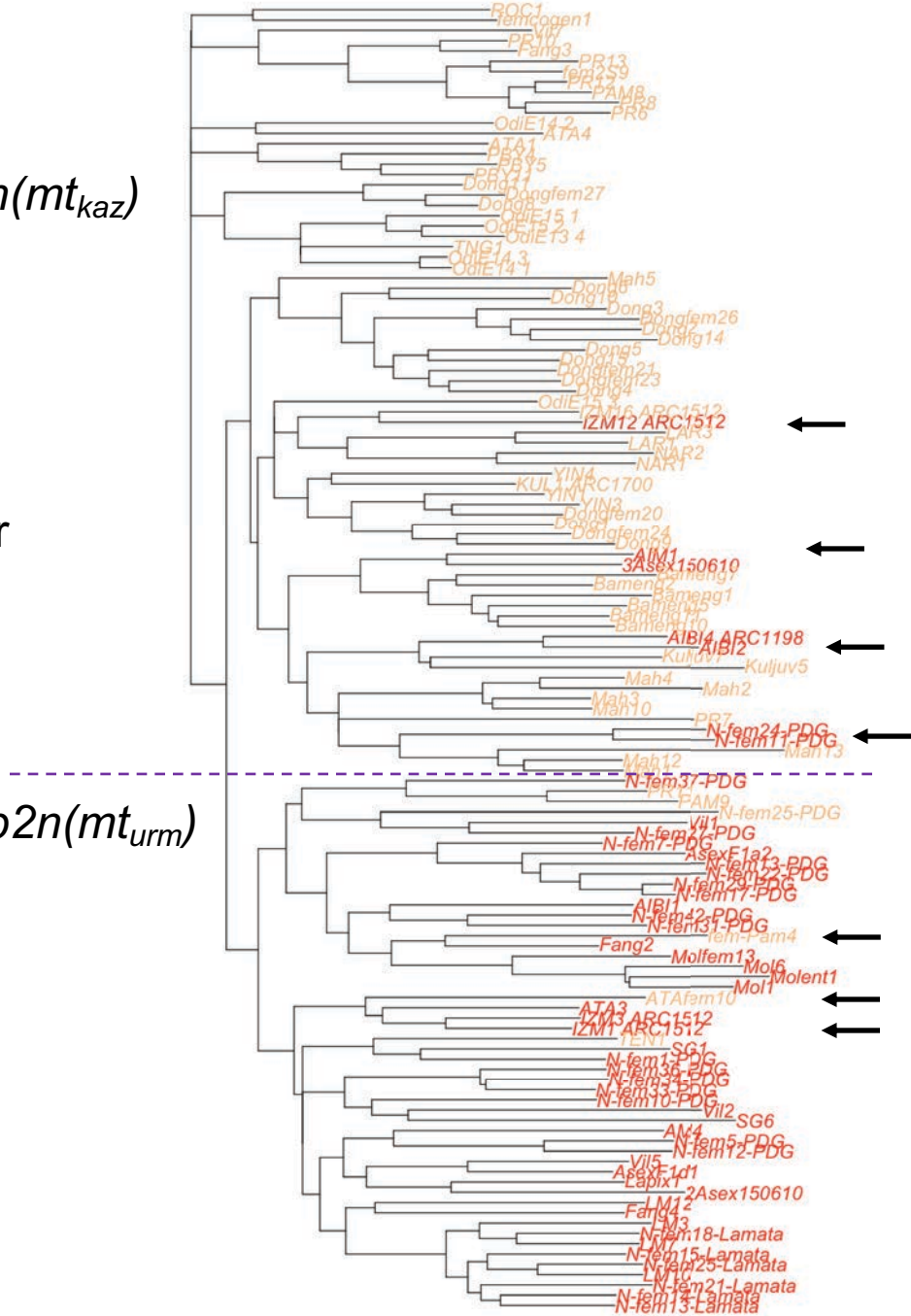
Application to *Ap2n*

Ap2n(mt$_{kaz}$)

Distance matrix between 127 individuals

NJ tree

➔ Significant association between nuclear genetic distance and mitochondrial haplotype (P<0.001)



Ap2n(mt$_{urm}$)

Application to *Ap2n*

Ap2n(mt$_{kaz}$)

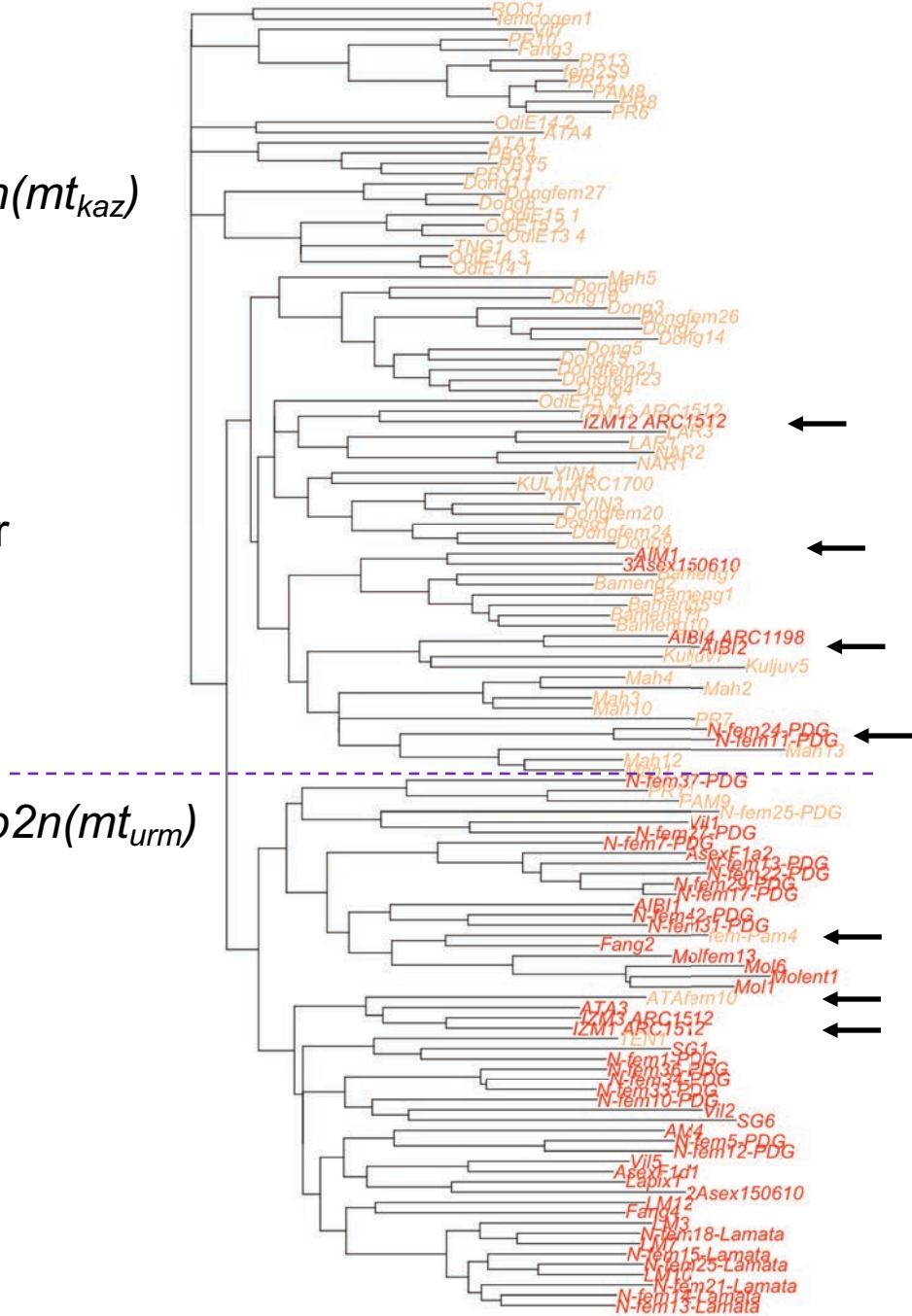
Distance matrix between 127 individuals

NJ tree

➔ Significant association between nuclear genetic distance and mitochondrial haplotype (P<0.001)

Three hypotheses:
random noise
rare events of sex among *Ap2n*
contagious asexuality

Ap2n(mt$_{urm}$)



Application to *Ap2n*

Ap2n(mt$_{kaz}$)

Distance matrix between 127 individuals

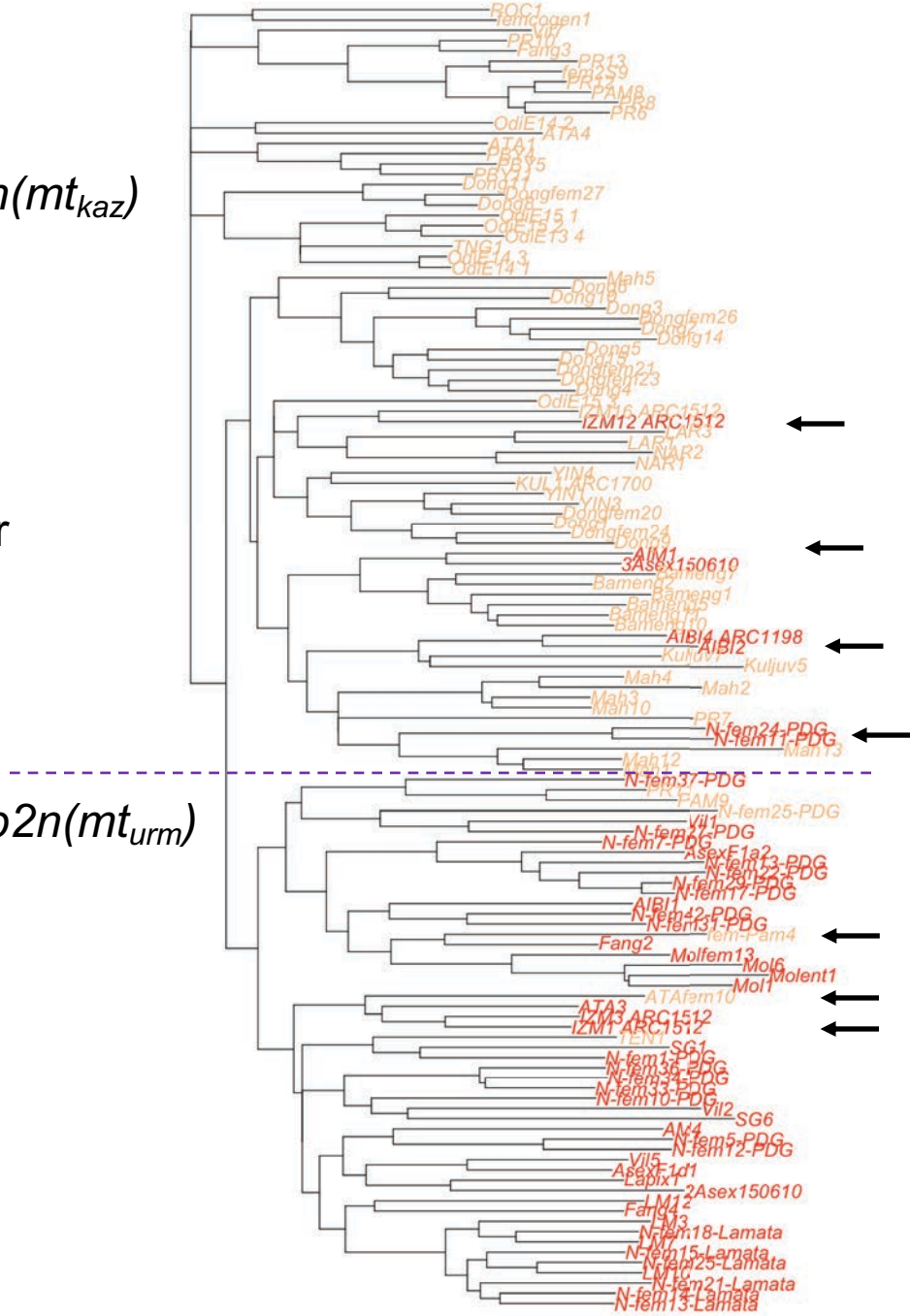
NJ tree

➔ Significant association between nuclear genetic distance and mitochondrial haplotype (P<0.001)

Three hypotheses:
random noise
rare events of sex among *Ap2n*
contagious asexuality

Shown in the lab (Boyer, unpublished)

Ap2n(mt$_{urm}$)



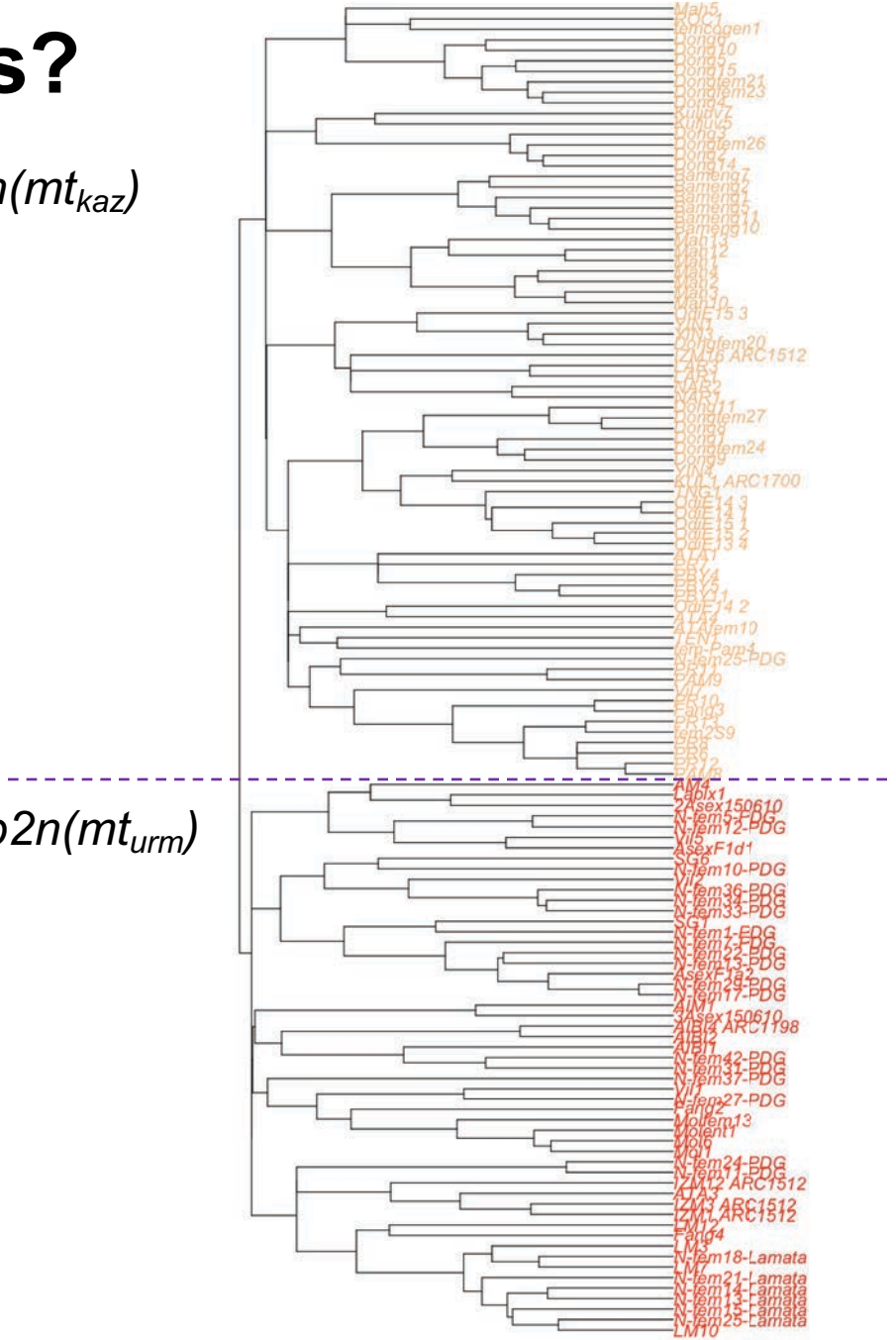
Monophyly of mt clades?

Branch length=15434.15

Bootstrap test:
1/ resampling of 12 loci
2/ NJ tree
3/ branch length

Ap2n(mt_{kaz})

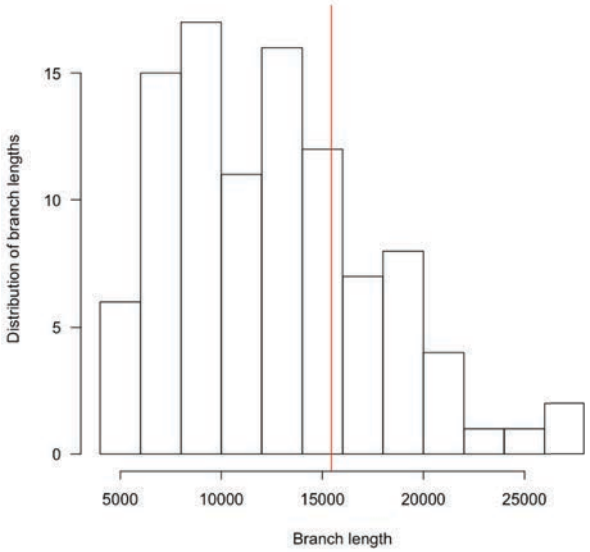
Ap2n(mt_{urm})



Monophyly of mt clades?

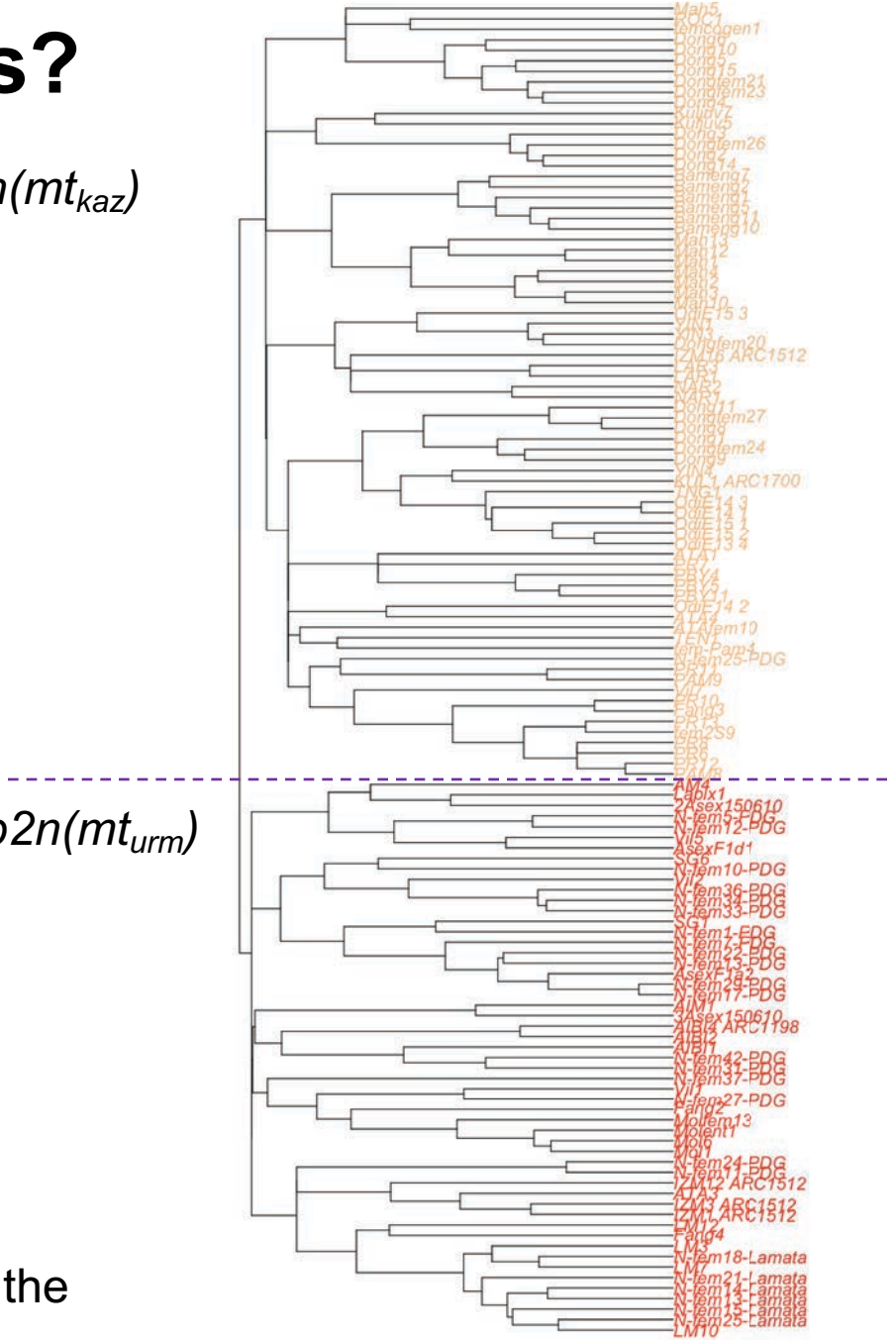
Branch length=15434.15

Bootstrap test:
1/ resampling of 12 loci
2/ NJ tree
3/ branch length



Ap2n(mt$_{kaz}$)

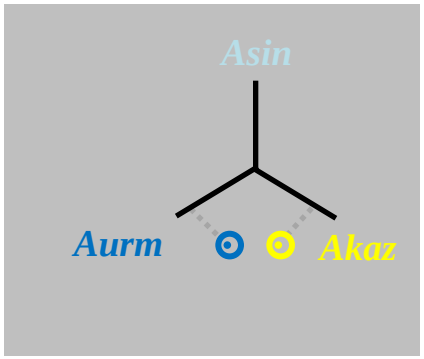
Ap2n(mt$_{urm}$)



Cannot rule out a monophyletic tree
=> No sex or sex only among clones with the same mt haplotype

Scenarios for the origin of *Ap2n*

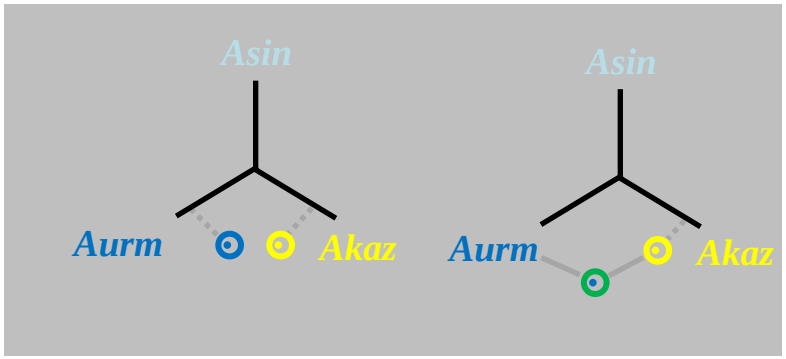
(A) Independent spontaneous origins



Scenarios for the origin of *Ap2n*

(A) Independent spontaneous origins

(B) Spontaneous origin and hybridization



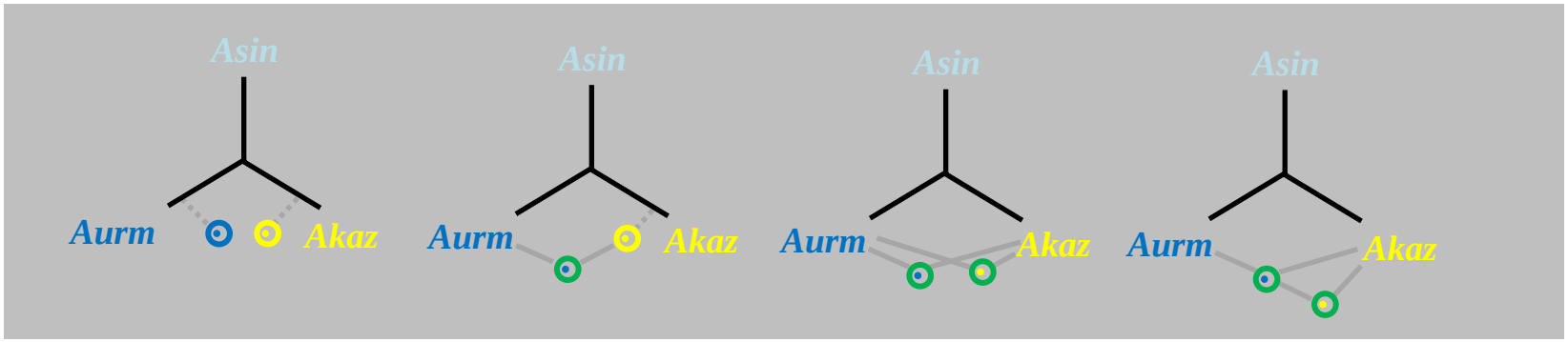
Scenarios for the origin of *Ap2n*

(A) Independent spontaneous origins

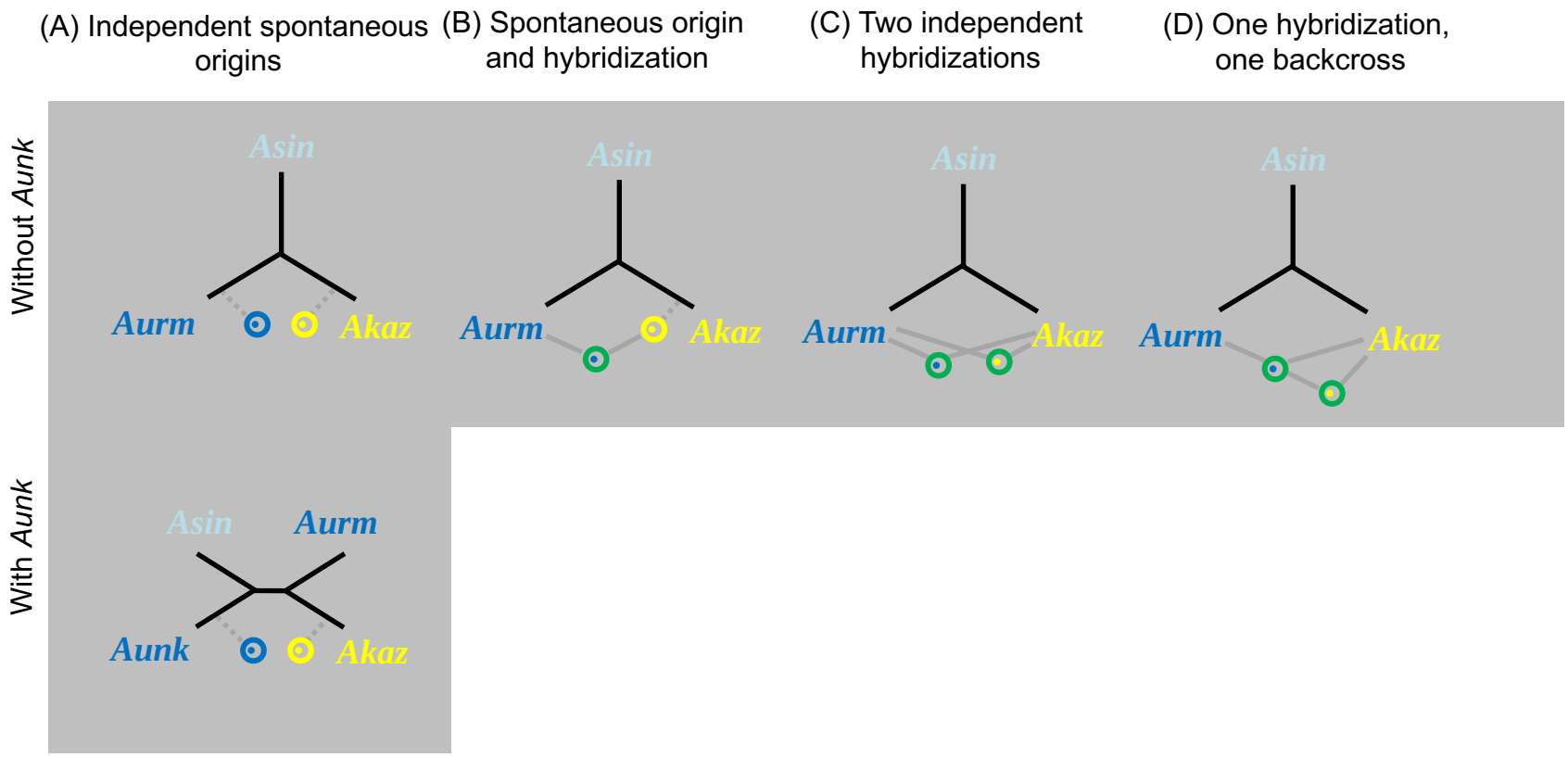
(B) Spontaneous origin and hybridization

(C) Two independent hybridizations

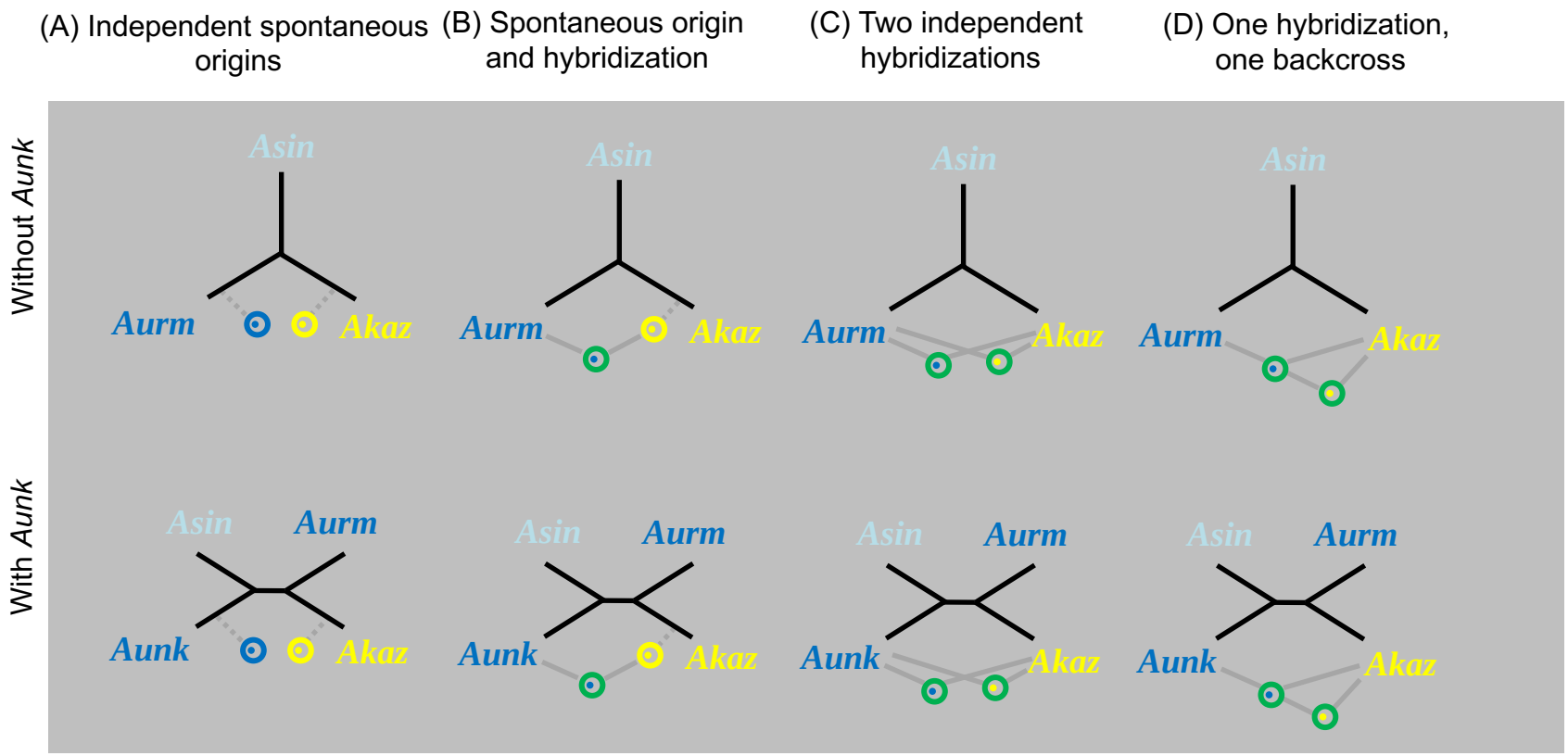
(D) One hybridization, one backcross



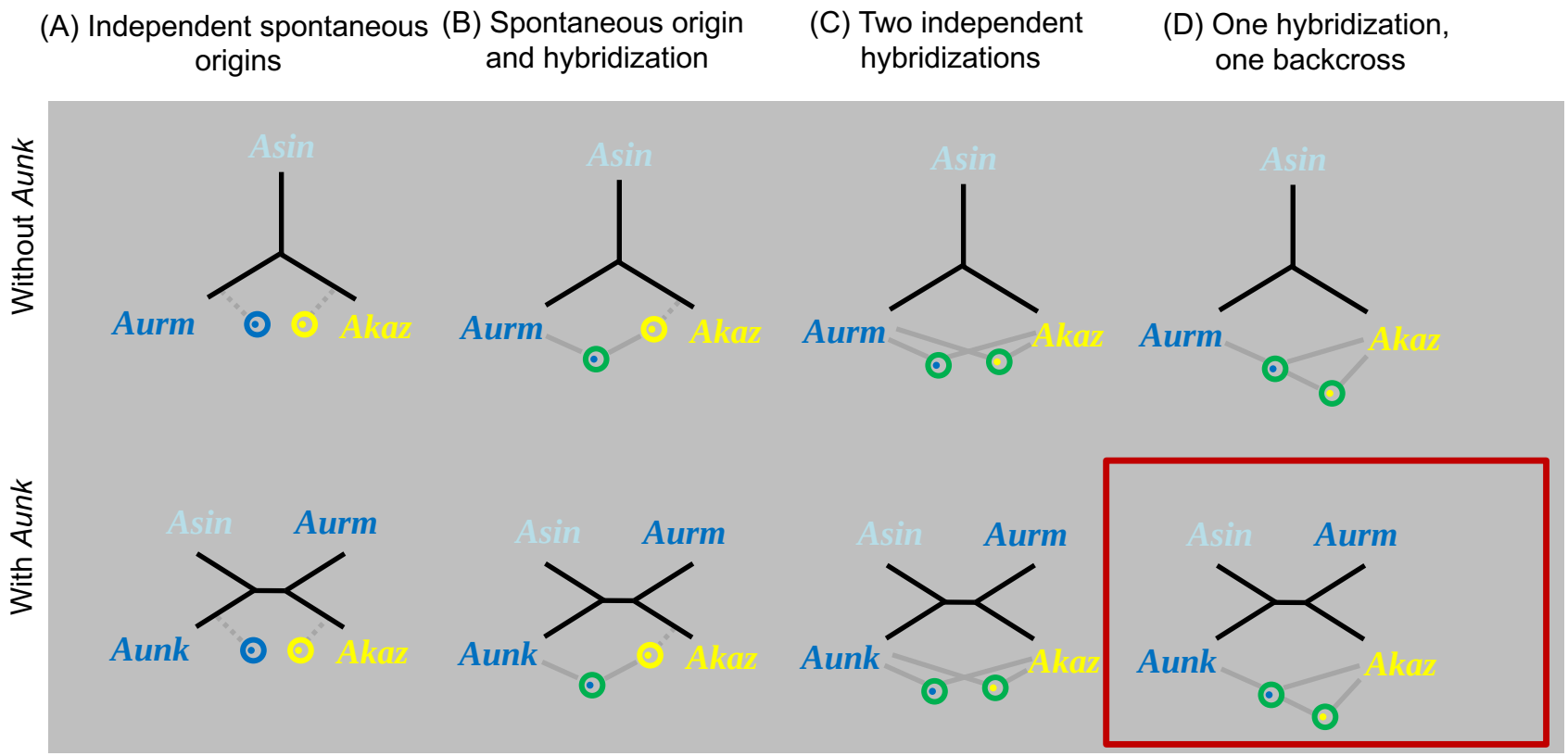
Scenarios for the origin of *Ap2n*



Scenarios for the origin of *Ap2n*



Scenarios for the origin of *Ap2n*



Other scenarios:
ΔAICc>2

Outline

1. Number of maternal origins and diversity levels in *Ap2n-5n*

   - Number of mitochondrial haplotypes
   - Genome size
   - Levels of nuclear diversity

2. Mode(s) of origin of *Ap2n*
   - Monophyly of clades based on mitochondrial haplotypes?
   - Comparison of different evolutionary scenarios

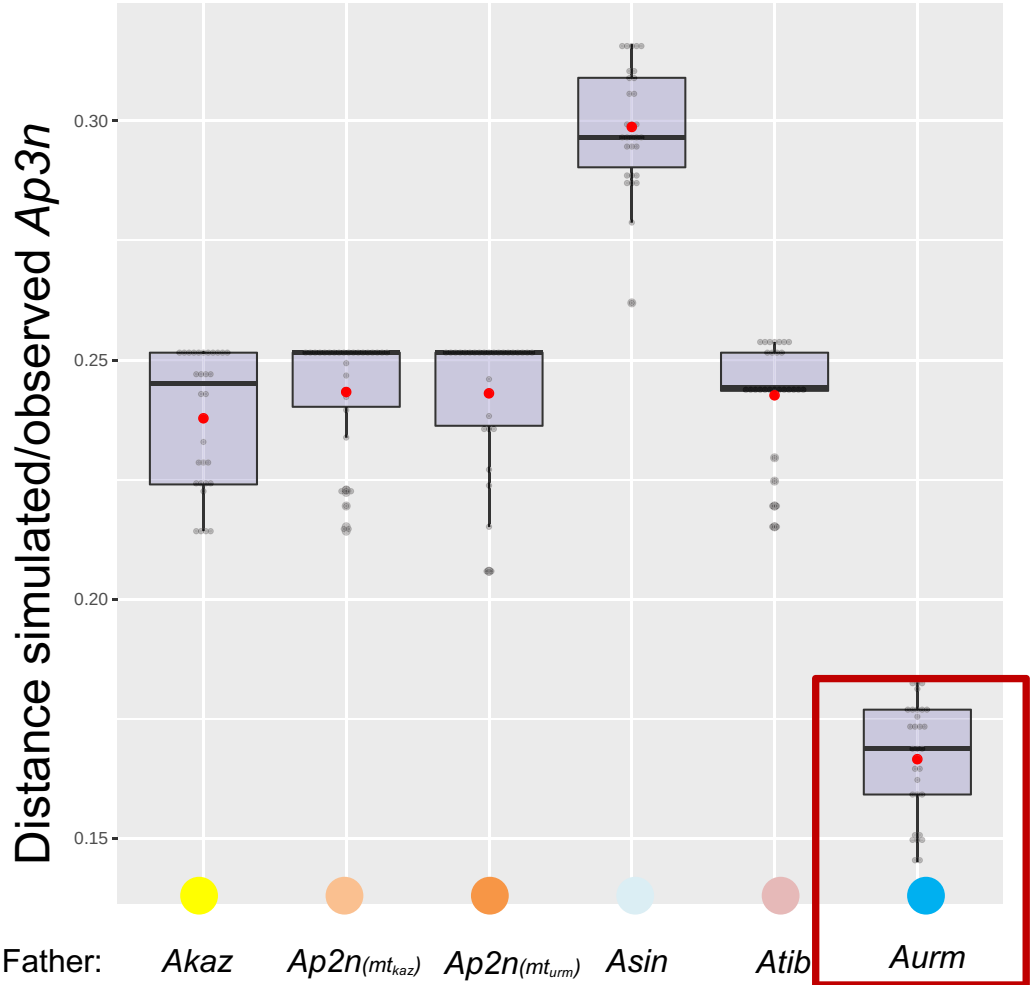
3. Mode(s) of origin of *Ap3n*, *Ap4n*, *Ap5n*

Scenarios for the origin of *Ap3n*

Simulations:
1/ Sample *Ap2n(mt$_{kaz}$)* mother genotype
2/ Sample male from sexual or
Ap2n genotype
3/ Create gamete
4/ Create hybrid
5/ Distance to *Ap3n*

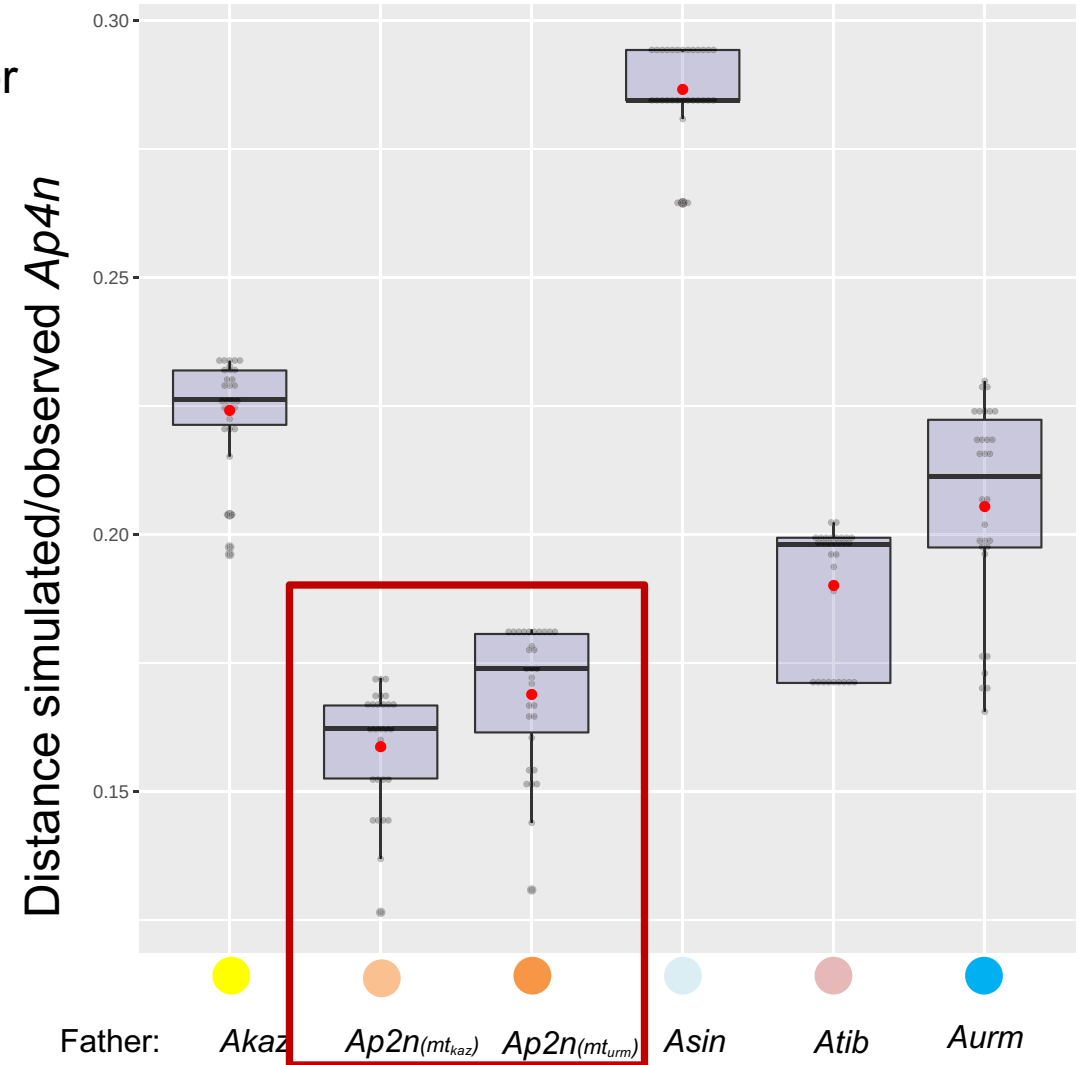
Scenarios for the origin of *Ap3n*

Simulations:
1/ Sample *Ap2n(mt$_{kaz}$)* mother genotype
2/ Sample male from sexual or
Ap2n genotype
3/ Create gamete
4/ Create hybrid
5/ Distance to *Ap3n*



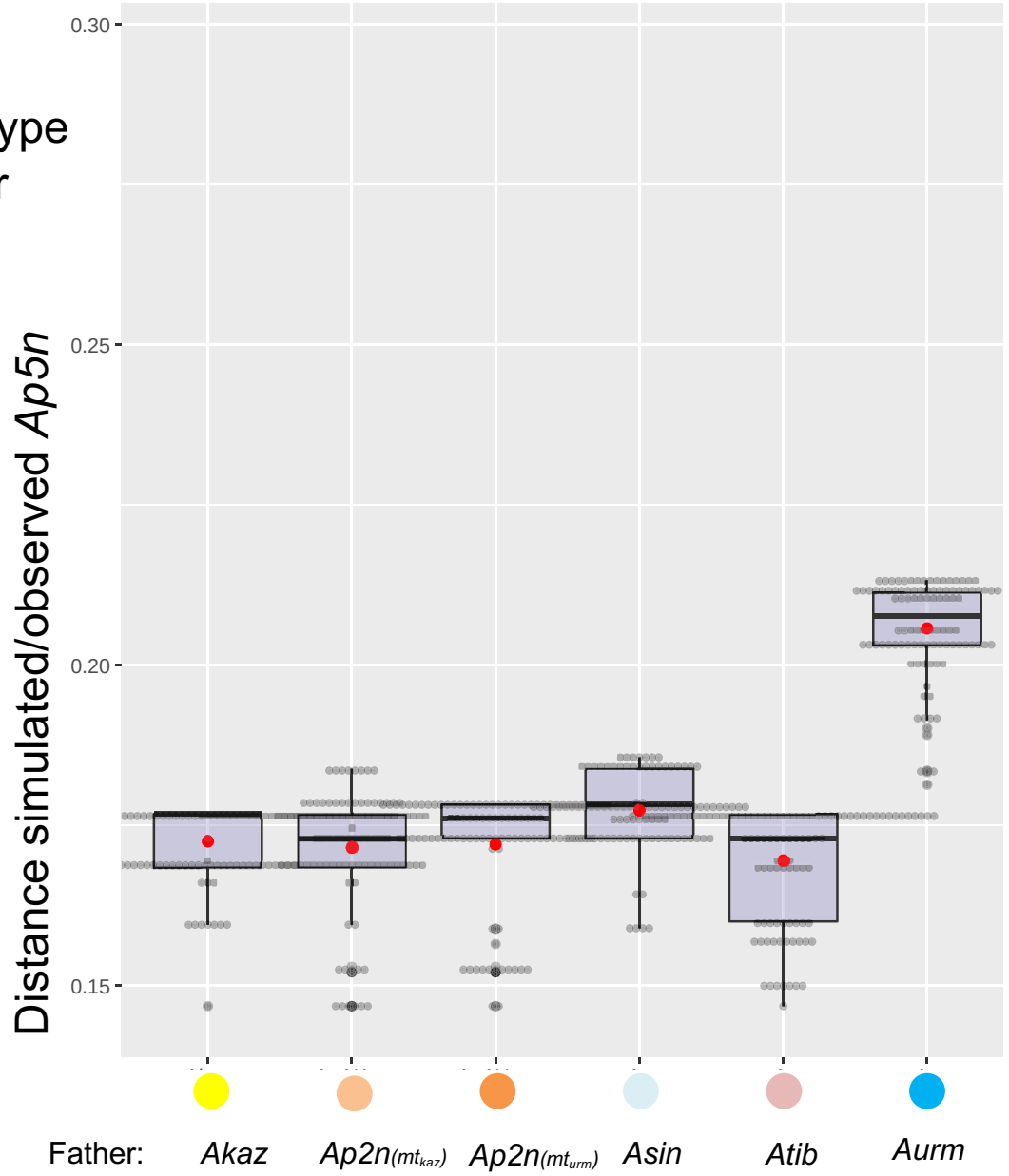
Scenarios for the origin of *Ap4n*

Simulations:
1/ Sample *Asin* mother genotype
2/ Create ovule
3/ Sample male from sexual or *Ap2n* genotype
3/ Create gamete
4/ Create hybrid
5/ Distance to *Ap4n*



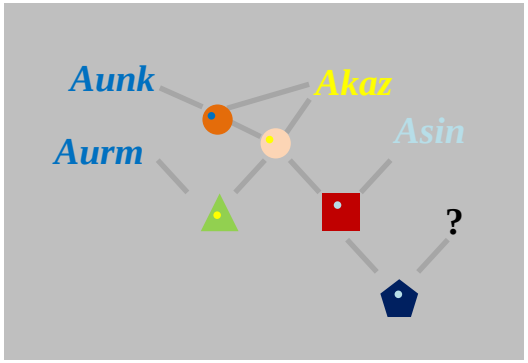
Scenarios for the origin of *Ap5n*

Simulations:
1/ Sample *Ap4n* mother genotype
2/ Sample male from sexual or *Ap2n* genotype
3/ Create gamete
4/ Create hybrid
5/ Distance to *Ap5n*



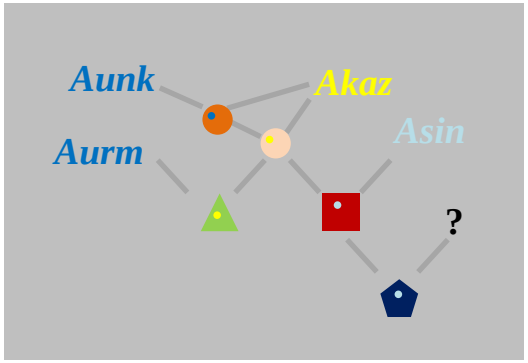
Conclusion

Multiple, but non-independent origins of *Artemia parthenogenetica*



Conclusion

Multiple, but non-independent origins of *Artemia parthenogenetica*

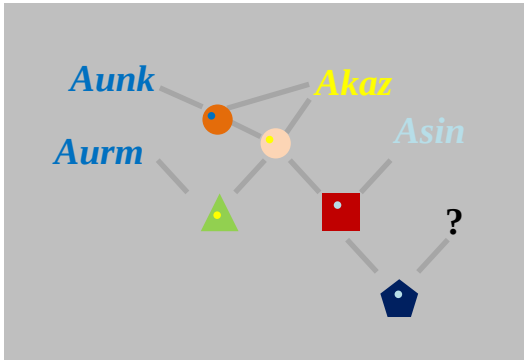


Barigozzi 1974

Revision of the taxonomy?

Conclusion

Multiple, but non-independent origins of *Artemia parthenogenetica*



Ap2n(mt$_{urm}$): AA+ZW
Ap2n(mt$_{kaz}$): AA+ ZW
Ap3n: AAA+ ZZW
Ap4n: AAAA+ ZZWW
Ap5n: AAAAA+ ZZZWW

Barigozzi 1974

Revision of the taxonomy?

Genetic determinism of asexuality?

Conclusion

Ancient origin of *Ap2n* with low but non zero recombination

Polyploids are derived from *Ap2n*
⇒ doubts on apomixis in polyploids
⇒ meiosis in polyploids?
⇒ preferential pairing with Z homologue
⇒ recombination, but no rare male

Ap2n(mt_{urm}*)*: ZW
Ap2n(mt_{kaz}*)*: ZW

Ap3n: ZZW
Ap4n: ZZWW
Ap5n: ZZZWW

Acknowledgements

Thomas Lenormand

Roula Zahab

Elodie Flaven

Advice and help:

Christoph Haag
Arnaud Estoup
Laure Benoit
France Dufresne
Paco Amat
Gilbert Van Stappen

INRA SCIENCE & IMPACT

Centre for Biology and Management of Populations
CBGP
Centre de Biologie pour la Gestion des Populations

CEFE-CNRS

UNIVERSITÉ MONTPELLIER 2
SCIENCES ET TECHNIQUES

erc