Ernst-Moritz-Arndt University Greifswald
Institute of Zoology

Genetical determination of the origin of Aquatic Warblers wintering in Senegal

Anna-Luise Vogel

September 2009

Material:

435 Aquatic Warblers from 11 breeding populations, samples taken in 1994-2004 (AWCT, Benedikt Giessing)

59 Aquatic Warblers from Senegal (Djoudj), winter 2006/2007 and 2007/2008 (AWCT)

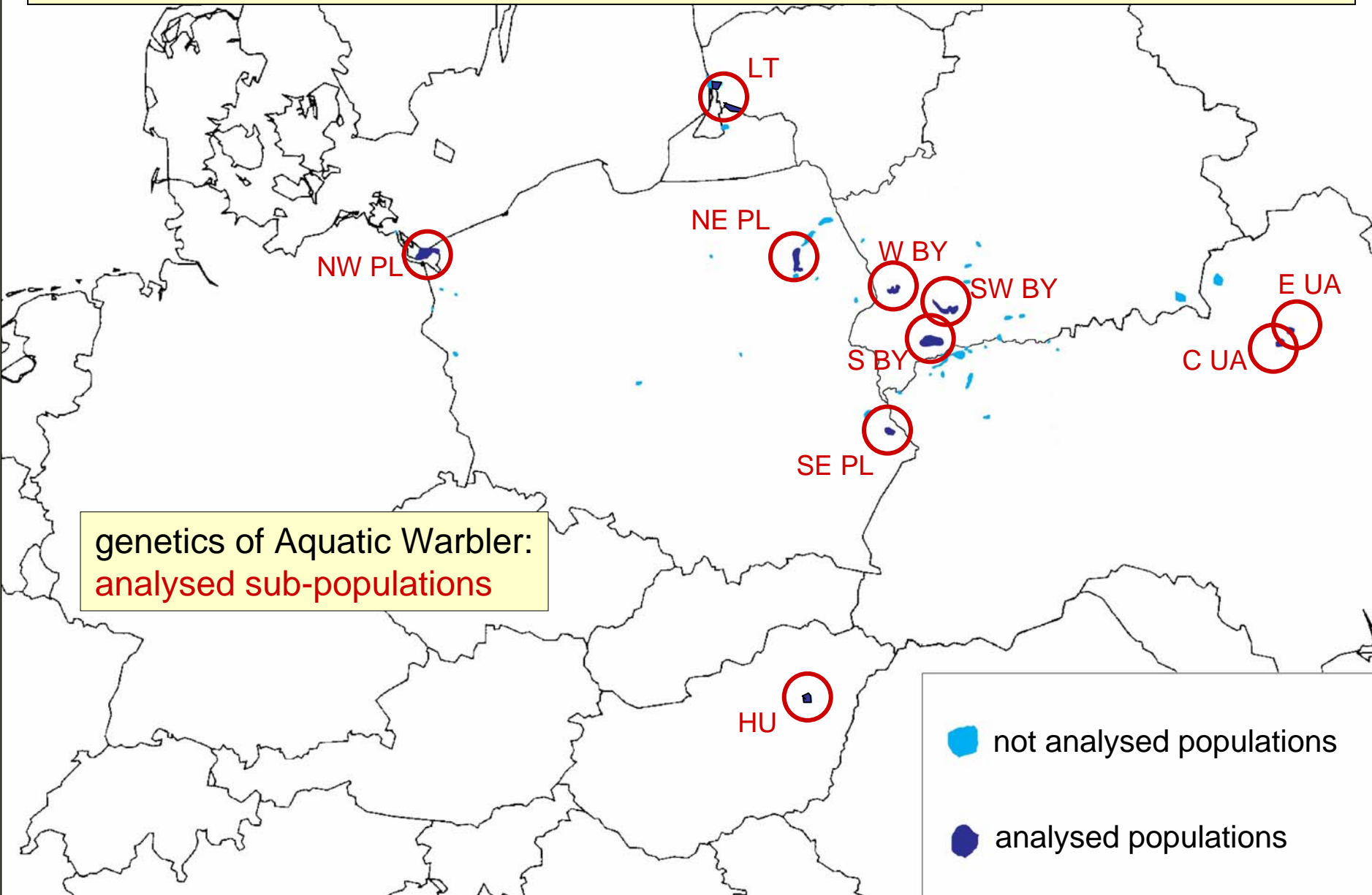(72 samples from Djoudj from 2009 not included yet)

Methods:

Investigation of 6 microsatellite loci, tested and selected by Giessing (2002)

Re-analysis of the ‚Giessing samples‘ with the same technique

Analysis with ‚*Structure 2.0*‘ and ‚*GeneClass 2.0*‘

Population genetic analyses of the Aquatic Warbler
Benedikt Gießing

LT

NE PL

NW PL

W BY

SW BY

S BY

E UA

C UA

SE PL

genetics of Aquatic Warbler:
analysed sub-populations

HU

not analysed populations

analysed populations

DNA samples from AW breeding populations, analysed by GIESSING (2002) and follow-up study, re-analysed by A. VOGEL 2009

Sampled AW population	year	individuals
Pomerania (Karsiborska Kepa), PL	1999	47
Biebrza, PL	1993, 1994, 1997	179
Chelm Marshes, PL	1998	30
Dikoe, BY	1999	49
Zvanets, BY	1999	30
Yaselda, BY	2000	27
Udaj, UA	2000	25
Supoj, UA	1999, 2000	25
Nemunas delta, LT	2003	10
Hortobágy, HU	2004	11
Omsk Oblast, Siberia, RU	2000	2
Total		**435**

Analysed Loci by B. GIESSING (2002) and A. VOGEL (2009)

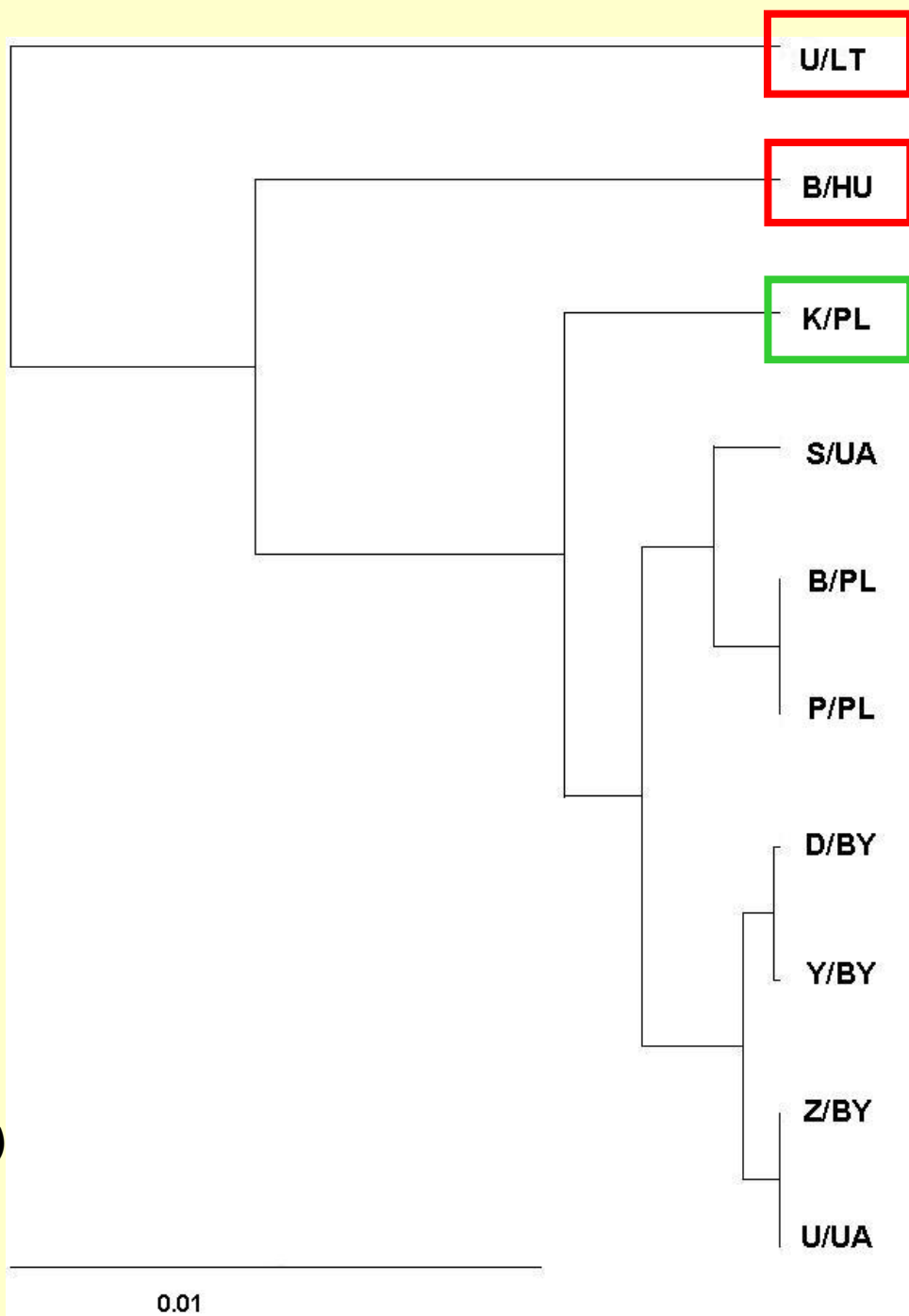| Locus	Annealing	Elongation	Zyklen	Sequenz	Quelle
AAR3	50° C, 20 s	15 s	45	5'-gca tct ggt ctc cga ttg tt-3' 5'-ttt ggg tta cat ctg agt gtg c-3'	Hannson et al. (2000)
FhU2	55° C, 30 s	20 s	40	5'-gca cag gta aat att tgc tgg gcc-3' 5'-gtg ttc tta aaa cat gcc tgg agg-3'	Primmer et al. (1996)
POCC8	52° C, 30 s	20 s	31	5'-gca tgt ctc ttc aga cat ctg c-3' 5'-atg tag agc tcc cat ggt gg-3'	Bensch et al. (1997)
ASE12	55° C, 30 s	30 s	40	5'-tca agg aaa cac aac tac agc c-3' 5'-ttt cct cac agc ctt gac tg-3'	Richardson et al. (2000)
ASE19	55° C, 30 s	20 s	40	5'-tct gcc cat tag gga aaa gtc-3' 5'-tag ggt ccc agg gag gaa g-3'	Richardson et al. (2000)
ASE26	66° C, 30 s	20 s	45	5'-gct ggc ctt gca aaa act tc-3' 5'-aac acc tcc ctg tcc ctg c-3'	Richardson et al. (2000)

Results

Breeding populations:

1. ‚*Structure*' does not identify a significant population structure (substructuring) in the whole sample; variability is low.

2. The sample was then grouped into +/- similar clusters according to F_{ST} values.

3. LT and HU populations had the biggest genetical differences to each other and the other subpopulations

4. Private alleles (2) occurred only in the Pomeranian population, but at a very low frequency (4 and 1 out of 47 birds)

5. Siberian birds (n = 2): no clear differences, no private alleles

   - 1 most similar to Pomeranian birds (7 out of 12 allele lengths similar)

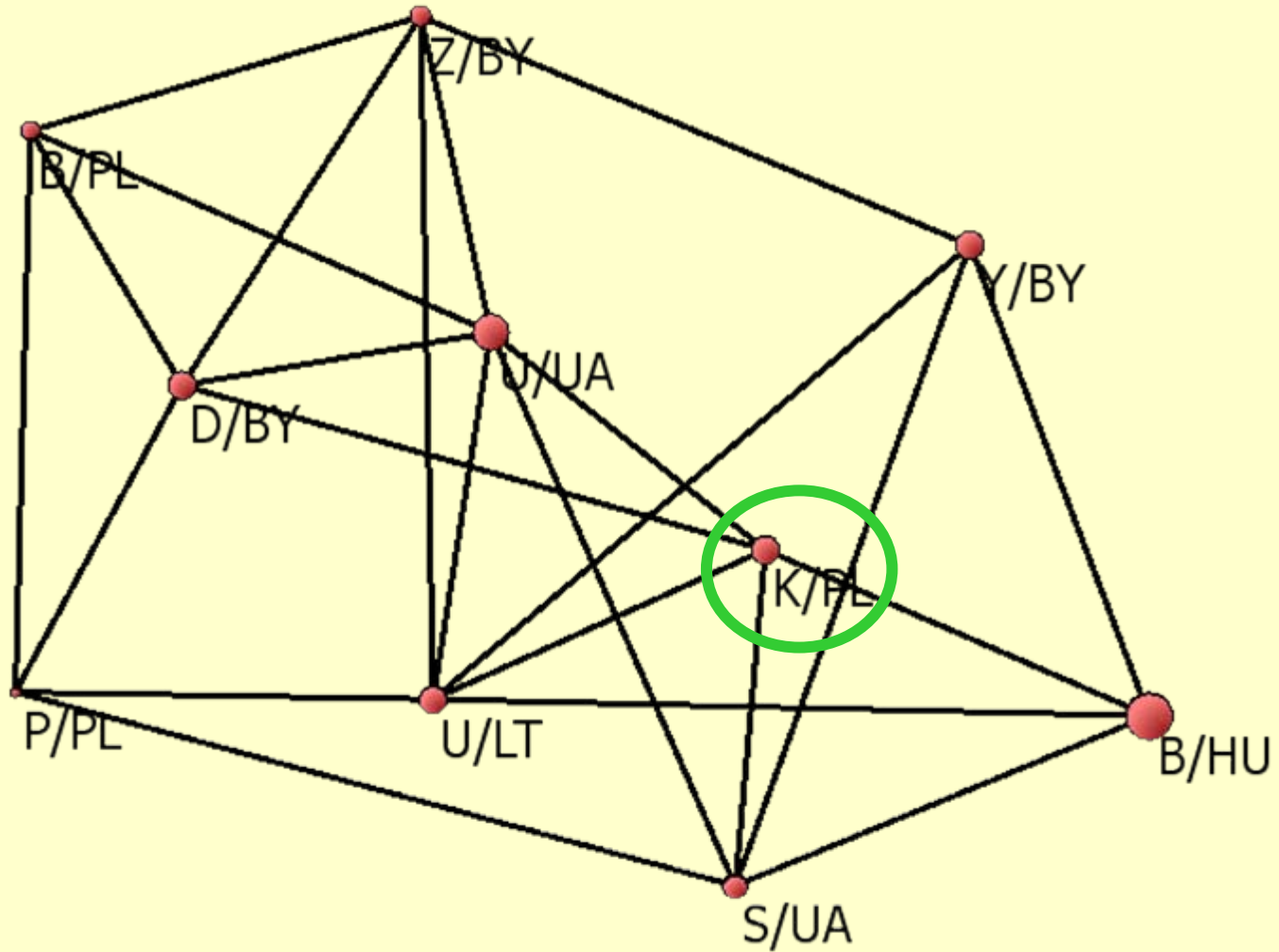   - 1 most similar to Cluster 2 birds (Belarus, Udaj/UA)

Dendrogramme (Neighbour-Joining)

U/LT

U/UA

Z/BY

Y/BY

D/BY

K/PL

P/PL

B/PL

S/UA

B/HU

0.01

Dendrogramme (UPGMA)

U/LT

B/HU

K/PL

S/UA

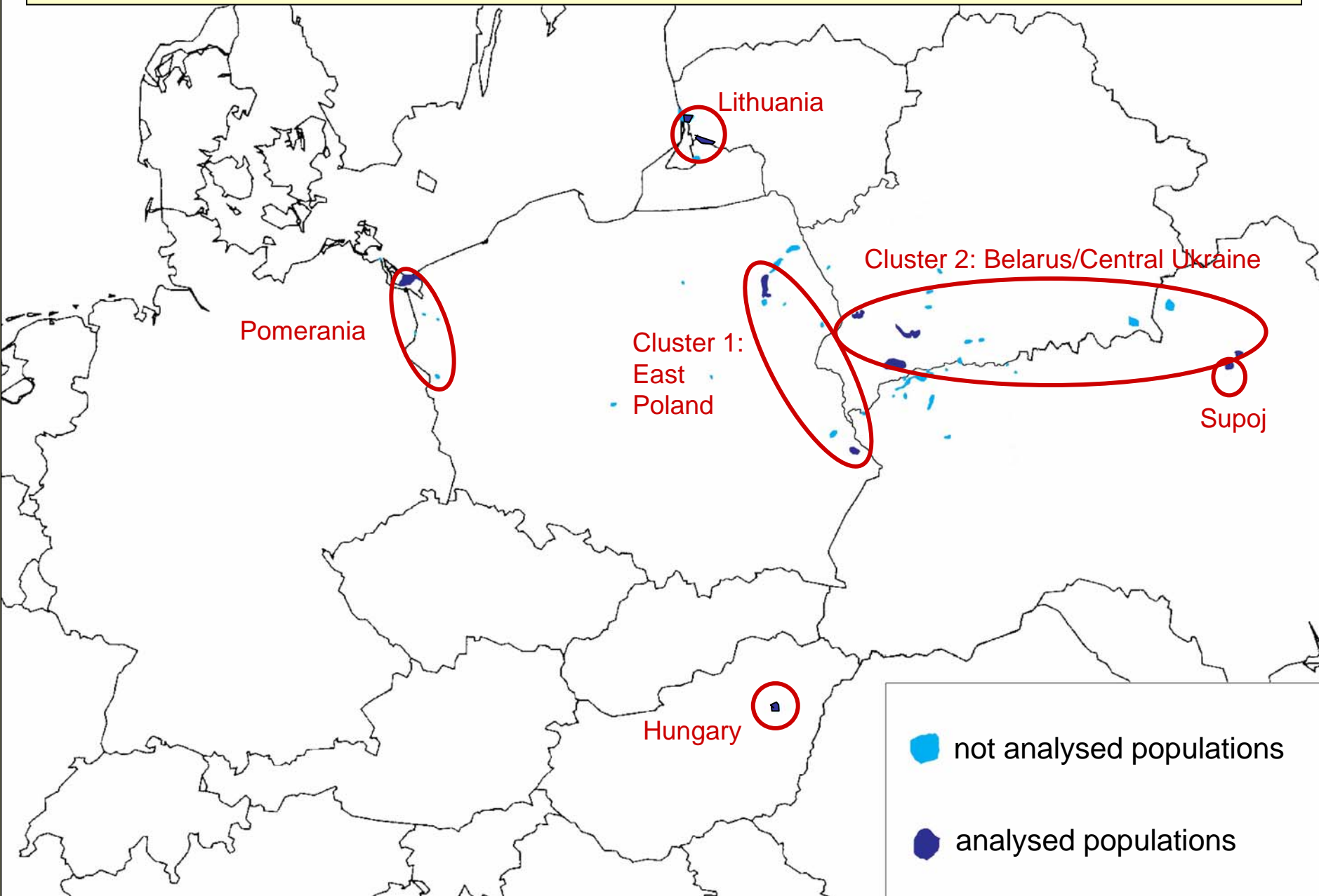B/PL

P/PL

D/BY

Y/BY

Z/BY

U/UA

0.01
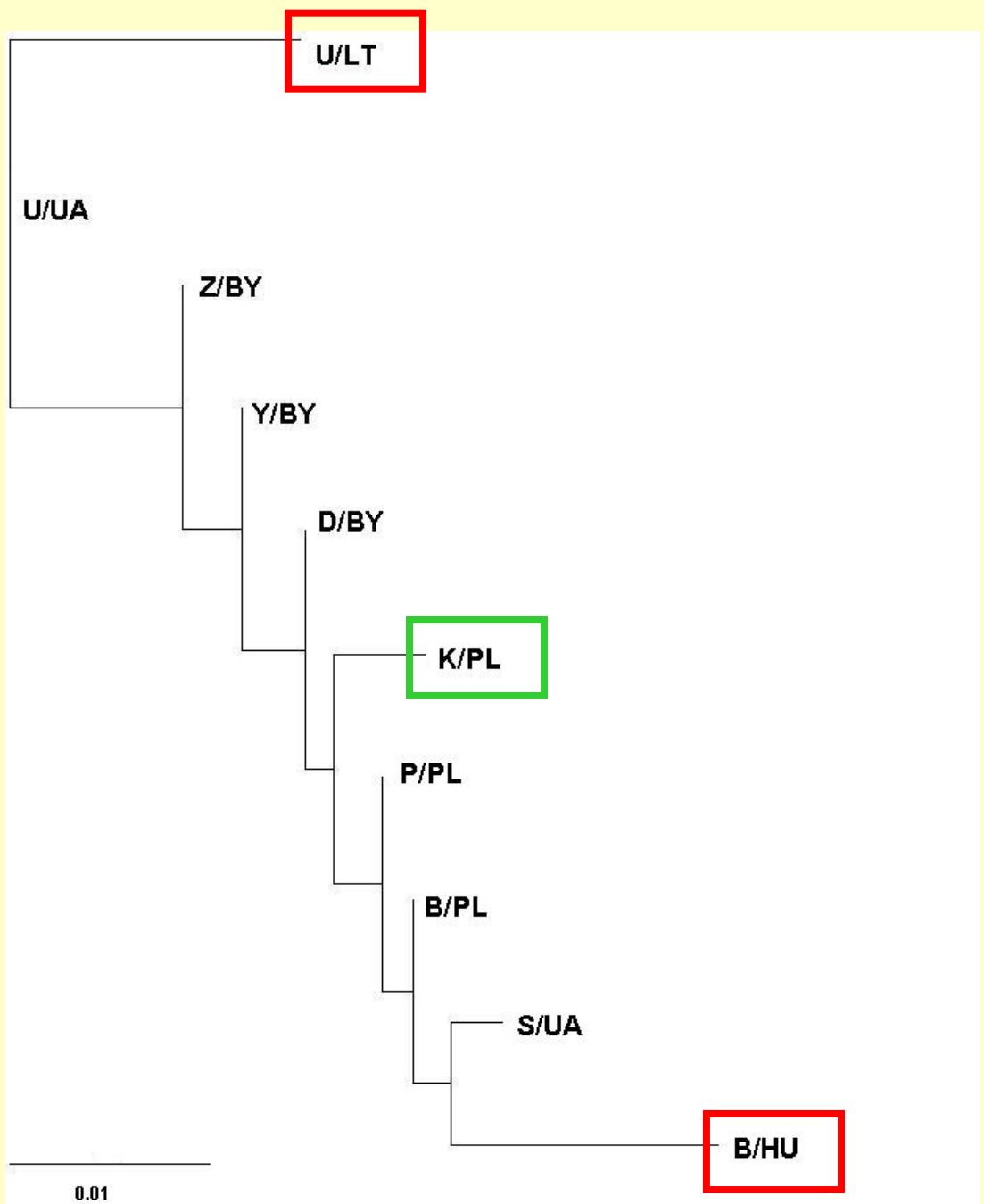
3D graph (, *PopulationGraph'*) of relationship between the AW populations. Size of red circles relates to the genetical diversity of populations.

New established population structure, according to F_{ST} values

abbreviation	population	individuals
K/PL	Pomerania	47
Cluster 1	E Poland: Biebrza, Chelm	209
Cluster 2	Belarus, Central Ukraine: Dikoe, Zvanets,Yaselda; Udaj	131
S/UA	South central Ukraine: Supoj	25
U/LT	Lithuania: Nemunas delta	10
B/HU	Hungary: Hortobágy	11

Population structuring (according to Anna Vogel**)**

Lithuania

Cluster 2: Belarus/Central Ukraine

Pomerania

Cluster 1:
East
Poland

Supoj

Hungary

not analysed populations

analysed populations

<u>Results</u>

**Genetical structure of AW captured in Djoudj:**

1. The 59 sampled indivuduals are likely to origin from different breeding populations:

   - Three (out of 6) loci were significantly outside the Hardy-Weinberg Equilibrium (increased homozygosity).

   - This relates to the loci with highest variability.

2. 'Structure' again does not identify any significant substructure

3. No previously unknown allele lengths (no indication for unknown breeding populations).
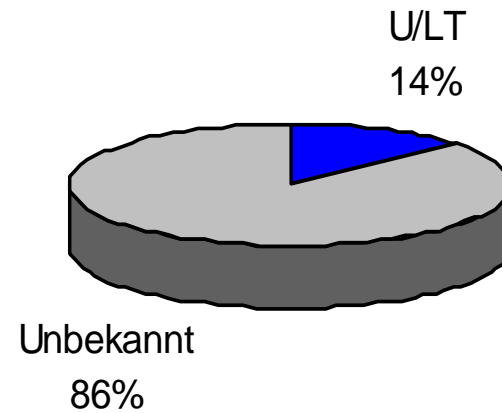
4. No private alleles.

Results

Assignment of Djoudj birds

1. Only very few <u>significant</u> assignments:
    5 loci used: 1 bird - Pomerania
    6 loci used: 2 birds - Lithuania

2. If reduced criteria are used (assignment of an individual to a certain population, if difference in probability value to the next population is >0.2):

    *   20 % of ind. assigned: 8 x Pomerania, 4 x Lithuania (5 loci)

5 loci

6 loci

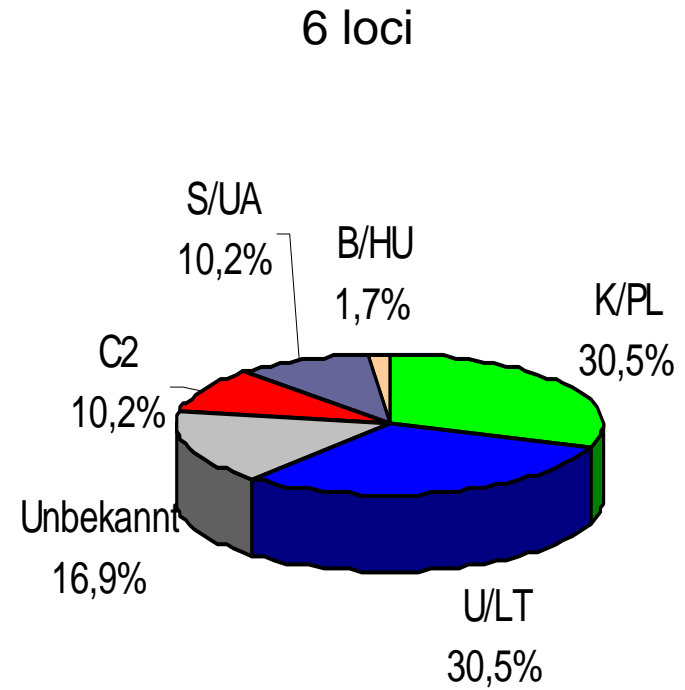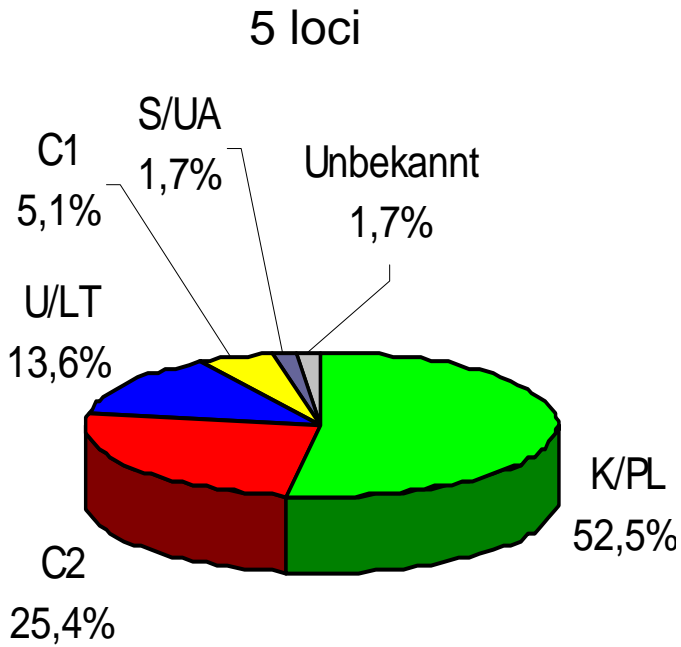K/PL
14% U/LT
7%

U/LT
14%

Unbekannt
79%

Unbekannt
86%
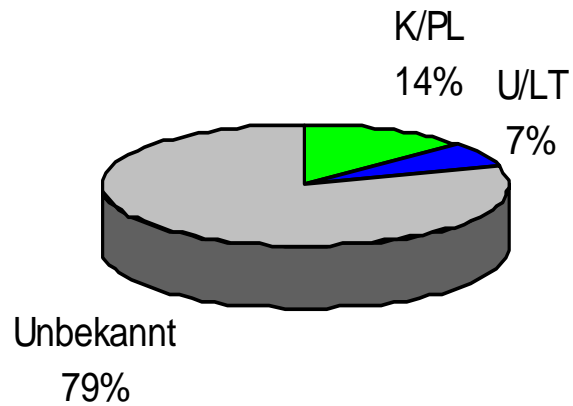
Assignment of Djoudj AW at a low probability level (difference to the next possible population >0.2 probabity value)

Results

Assignment of Djoudj birds

1. Only very few <u>significant</u> assignments:
   5 loci used: 1 bird - Pomerania
   6 loci used: 2 birds - Lithuania

2. If reduced criteria are used (assignment of an individual to a certain population, if difference in probability value to the next population is >0.2):

   - 20 % of ind. assigned: 8 x Pomerania, 4 x Lithuania

3. If further reduced criteria are uses (probability of >0.1 accepted):

   - 98.3 % of ind. assigned:
        52.5 % Pomerania
        25.4 % Belarus/Udaj
        13.6 % Lithuania

5 loci

- S/UA 1,7%
- C1 5,1%
- Unbekannt 1,7%
- U/LT 13,6%
- K/PL 52,5%
- C2 25,4%

6 loci

- S/UA 10,2%
- B/HU 1,7%
- C2 10,2%
- K/PL 30,5%
- Unbekannt 16,9%
- U/LT 30,5%

Assignment of Djoudj AW at a <u>very low</u> probability level (>0.1)

Results

Assignment of Djoudj birds

1. Only very few <u>significant</u> assignments:
    5 loci used: 1 bird - Pomerania
    6 loci used: 2 birds - Lithuania

2. If reduced criteria are used (assignment of an individual to a certain population, if difference in probability value to the next population is >0.2):

    *   20 % of ind. assigned: 8 x Pomerania, 4 x Lithuania

3. If further reduced criteria are uses (probabilty of >0.1 accepted):

    *   98.3 % of ind. assigned:
                52.5 % Pomerania
                25.4 % Belarus/Udaj
                13.6 % Lithuania

4. Result is not reliable because:

    *   Mis-relation of 100 + 300 birds in Pomerania and Lithuania versus 2,000-10,000 birds in Djoudj

    *   >150 birds ringed in Djoudj 2007-2009, but no recovery in Pomerania.

Results

Assignment of Djoudj birds

1. Only very few <u>significant</u> assignments:
    5 loci used: 1 bird - Pomerania
    6 loci used: 2 birds - Lithuania

2. If reduced criteria are used (assignment of an individual to a certain population, if difference in probability value to the next population is >0.2):

    • 20 % of ind. assigned: 8 x Pomerania, 4 x Lithuania

3. If further reduced criteria are uses (probabilty of >0.1 accepted):

    • 98.3 % of ind. assigned:
                52.5 % Pomerania
                25.4 % Belarus/Udaj
                13.6 % Lithuania

4. Result is not reliable because:

    • Mis-relation of 100 + 300 birds in Pomerania and Lithuania versus 2,000-10,000 birds in Djoudj

    • >150 birds ringed in Djoudj 2007-2009, but no recovery in Pomerania.

5. Hungarian population seems not to be found (yet) in Djoudj

Conclusions

1. Six (five) loci are not sufficient! => 10-12 recommended

2. 30 samples from each population recommended

3. That means at least 3 more years of work with high efforts (catching of birds, identification of best alleles, analysis work)