SUPPLEMENTARY INFORMATION

1. GENOTYPING

A. Genetic assignment of sex

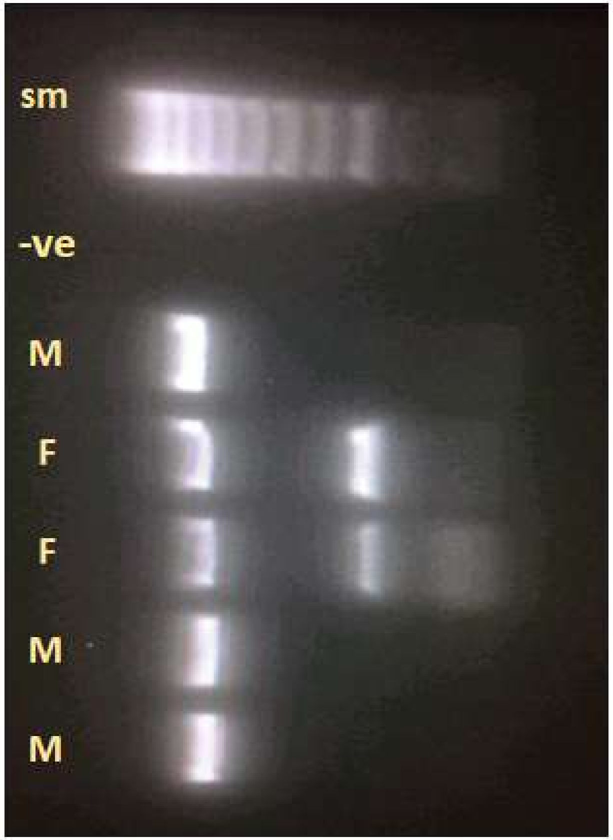


Figure SI 1A Genetic assignment of sex in adders
Agarose gel electrophoresis of products of duplex PCR, showing BDNF (autosomal) derived band in all samples, and W-specific band in females only.
PCR in Type-it mastermix (Qiagen), with primers CTNNB1W-F, CTNNB1-R, BDNF-F and BDNF-R (Laopichienpong et al 2017), each at 0.2 µM. Initial denaturation 95^{0}C 5 min, 35 cycles of 94^{0}C 30 sec, 55^{0}C 90 sec, 72^{0}C 90 sec, and final extension 72^{0}C 10 min.
sm: size marker 1kb ladder; -ve: template negative control; M: male; F: female

Laopichienpong, N., Tawichasri, P., Chanhome, L., Phatcharakullawarawat, R., Singchat, W., Kantachumpoo, A., … Srikulnath, K. (2017). A novel method of caenophidian snake sex identification using molecular markers based on two gametologous genes. *Ecology and Evolution*, *7*(13), 4661–4669. http://doi.org/10.1002/ece3.3057

Table SI 1B   Microsatellite loci used in study
Primers from reference a were derived in V.ursinii, those from b and c in V.berus.
Alleles NRS: total number of alleles per locus in study sample

locus	ref	repeat	anneal T^0	alleles NRS
Vu57	a	2	60	2
Vu4	a	3	60	5
CA71	c	2	60	2
Vb-B'2	b	2	58	3
CA11	c	2	60	2
CA3	c	2	58	2
Vb-B10	b	2	58	2
Vb-D17	b	3	58	3

ref a: Metzger et al (2011)b: Ursenbacher et al (2009a); c: Carlsson et al (2003
repeat: size of microsatellite; repeat motif (nucleotides); anneal T^0: PCR annealing temperature ^{0}C

PCR was performed in simplex in 10 µl volumes with 20–100 ng DNA, 5 µl mastermix (HotStarTaq Plus or Multiplex; Qiagen), 5 µmol/L unlabelled reverse primer and 5 µmol/L fluorophore-labelled forward primer (Applied Biosystems), with
initial denaturation 95°C 5 min, 60 cycles of 94°C 60 sec, 58-60°C 60 sec, 72°C 60 sec, and final extension 72°C 7 min, optimized in preliminary studies for each primer pair.

References:

•Metzger, C., Ferchaud, A. L., Geiser, C., & Ursenbacher, S. (2011). New polymorphic microsatellite markers of the endangered meadow viper (*Vipera ursinii*) identified by 454 high-throughput sequencing: When innovation meets conservation. *Conservation Genetics Resources*, *3*(3), 589–592. http://doi.org/10.1007/s12686-011-9411-x
•Ursenbacher, S., Monney, J. C., & Fumagalli, L. (2009). Limited genetic diversity and high differentiation among the remnant adder (*Vipera berus*) populations in the Swiss and French Jura Mountains. *Conservation Genetics*, *10*(2), 303–315. http://doi.org/10.1007/s10592-008-9580-7
•Carlsson, M., Isaksson, M., Hoggren, M., & Tegelstrom, H. (2003). Characterization of polymorphic microsatellite markers in the adder, *Vipera berus*. *Molecular Ecology Notes*, *3*, 73–75. https://doi.org/10.1046/j.1471-8286

SUPPLEMENTARY INFORMATION 2 ABC modelling of demographic history of study population

DIY ABC: method details

- Default settings were used for the microsatellite mutation priors, based on the generalized stepwise model (Di Rienzo et al.1994; Estoup, et al 2002). All loci were treated as a single group.
- A minimum of 10^5 simulations was performed per scenario. Scenarios were compared using linear discriminant analysis of summary statistics with logistic regression analysis (Beaumont 2008; Fagundes et al 2007) for estimation of posterior probability with 95% confidence intervals (Cornuet et al 2008).
- Models were evaluated for goodness of fit and potential discrepancies (Gelman et al 1995), with the inclusion of a range of summary statistics not used for the original simulation process.
- Parameters were estimated from the posterior parameter distributions of the 1% simulated datasets closest to the observed, using logit transformation.

Table SI 2.1

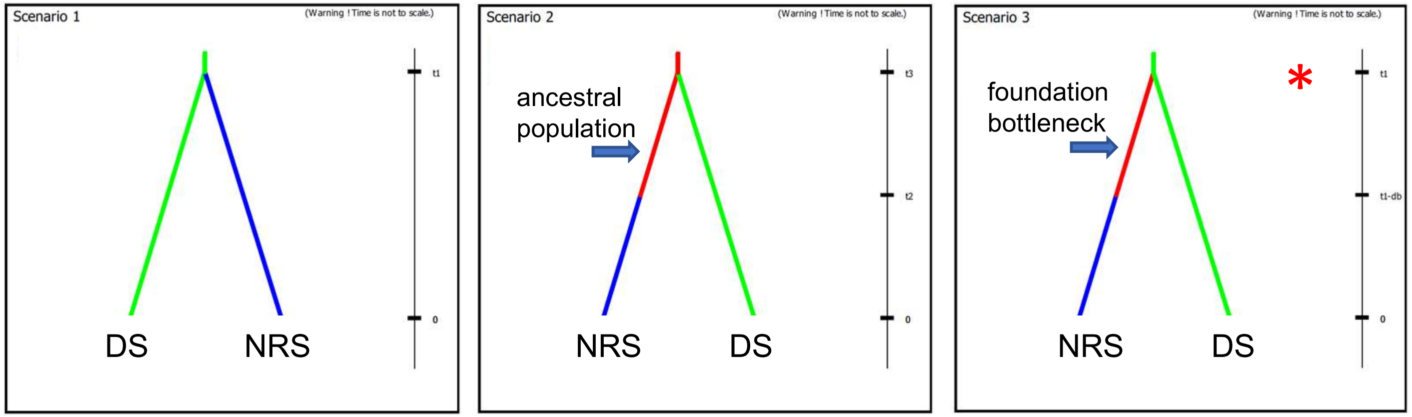
		Single sample statistics		Two sample statistics
For simulations	i)	mean number of alleles across loci	i)	mean number of alleles across loci (two samples)
	i)	mean gene diversity across loci (1)	i)	mean gene diversity across loci (two samples)
	i)	mean allele size variance across loci	i)	mean allele size variance across loci (two samples)
			i)	F_{ST} between two samples (3)
Model checking	i)	mean M index across loci (2)	i)	mean index of classification (two samples) (4)
			i)	shared allele distance between two samples (5)
			i)	$(\delta\mu)^2$ distance between two samples (6)

Table SI.2.1 Summary statistics used for ABC scenario simulations and model checking

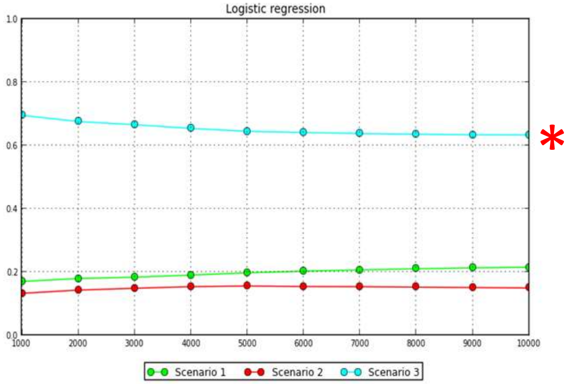
1. Nei (1987) 2. Garza and Williamson (2001); Excoffier et al (2005) 3. Weir & Cockerham, (1984) 4. Rannala & Mountain (1997); Pascual et al (2007) 5. Chakraborty & Jin (1993) 6. Goldstein et al (1995)

Figure SI 2.1 1/2 NRS Demography I: support for origin of reserve site population as founder event from donor site

Comparison of competing scenarios 1/2



0.2158 [0.1872,0.2444]	0.1504 [0.1293,0.1716]	0.6338 [0.5990,0.6686]



Schematic representation of competing scenarios in DIY ABC

1. Divergence of reserve site (NRS) and donor (DS) populations at time t1
2. Independent origin of NRS and donor populations from a common ancestral population at times t2 and t3 respectively
3. Origin of NRS from donor population at t1 with a foundation bottleneck

Top right panel: posterior probabilities for the 3 scenarios, showing scenario 3 to be most strongly supported (probability and 95% CI shown below relevant scenario)

Bottom right panel: model fit plot of first two principal components in PCA plot, placing the location of the observed dataset (yellow circle) within the cloud of simulated posteriors (outlined circles). Priors are shown as smaller pale circles.

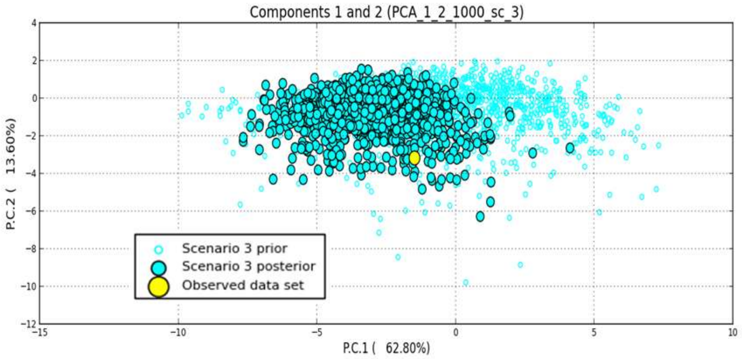
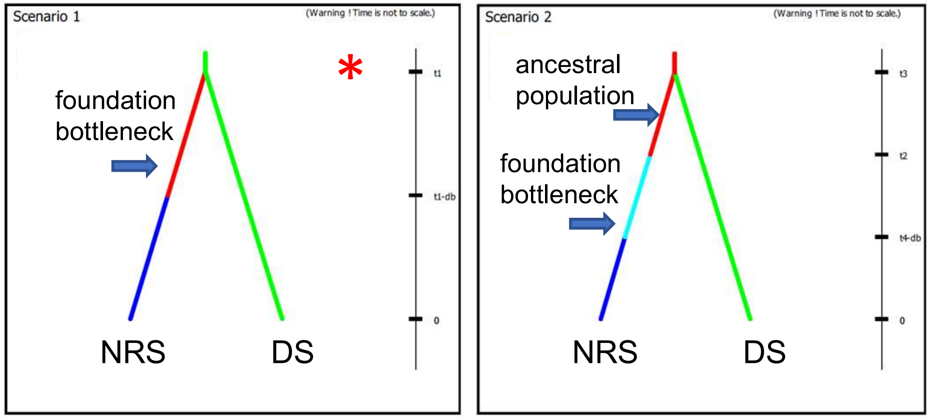
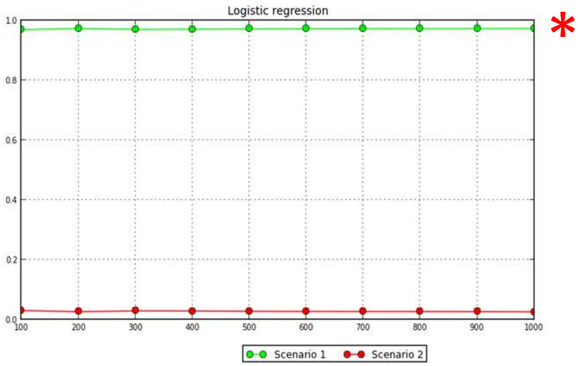


Figure SI 2.1 2/2

Comparison of competing scenarios 2/2



0.9730 [0.9624,0.9835]	0.0270 [0.0165,0.0376]



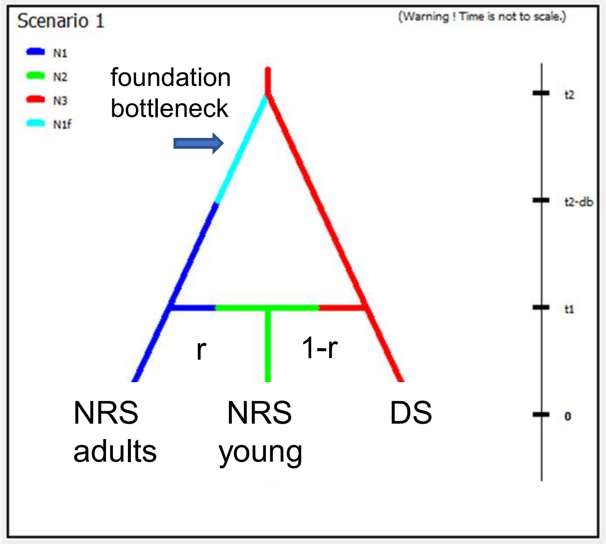
Comparison of supported scenario from comparison 1 with scenario of independent origin of NRS and DS from a common ancestral population, incorporating a foundation bottleneck for NRS. The model of the reserve site originating from the donor population with a foundation bottleneck is again strongly supported.

	parameter	prior
N NRS across scenario	N1	u[10,10^4]
N DS across scenario	N2	u[10,10^4]
N ancestral population	N3	u[10,10^4]
origin NRS from DS	t1	u[5,500]
origin NRS from ancestral	t2	u[5,500]
origin DS from ancestral	t3	u[5,500]
duration bottleneck	db	5
N during bottleneck	Nf1	u[2,50]

Prior parameters for simulations
Times are in generations before time of sampling (t0)
u: uniform
db: duration of bottleneck (fixed at five generations)

Figure SI 2.2 1/2 NRS demography II: support for additional translocation from donor site

A



B

r prior	probability [0.95% CI]
u [0 - 0.5]	0.0120 [0.0057,0.0183]
u [0.5 - 1]	0.9880 [0.9817,0.9943]
r = 1	0.4190 [0.3843,0.4536]
u [0.8 – 0.99]	0.5810 [0.5464,0.7050]
r = 1	0.3318 [0.2950,0.3686]
r = 0.9	0.6682 [0.6314,0.7050]

A Comparison of competing scenarios

Scenario in which the young individuals of NRS are derived from admixture between NRS adults and the original donor population at time t1, where r is the proportion derived from NRS, and 1-r derived from DS.

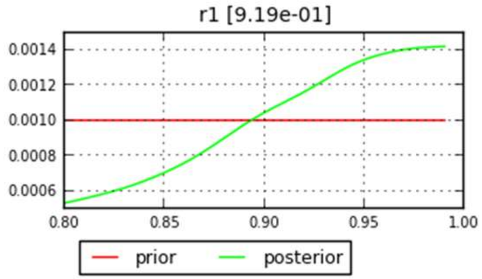
The scenario incorporates the previously supported model in which NRS is derived from DS with a foundation bottleneck at time t2. To ensure equivalent complexity between competing scenarios, competing scenarios had the same format, with different prior values for r.

B. Posterior probabilities for competing scenarios

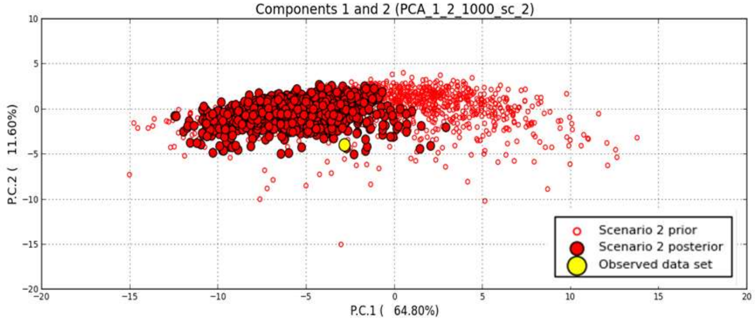
Panels showing posterior probabilities for pairs of competing scenarios, with progressive narrowing of priors for r. Values for the supported scenario are highlighted. The lower panel shows the scenario with a fixed value for r of 0.9 to be supported over a model in which there is no input from the donor site, where r =1 (fixed)

Figure SI.2.2 2/2

C



D



C. Parameter distribution for r from scenario with prior U[0.8-0.99]. The modal distribution relative to priors indicates influence of genetic factors on distribution.

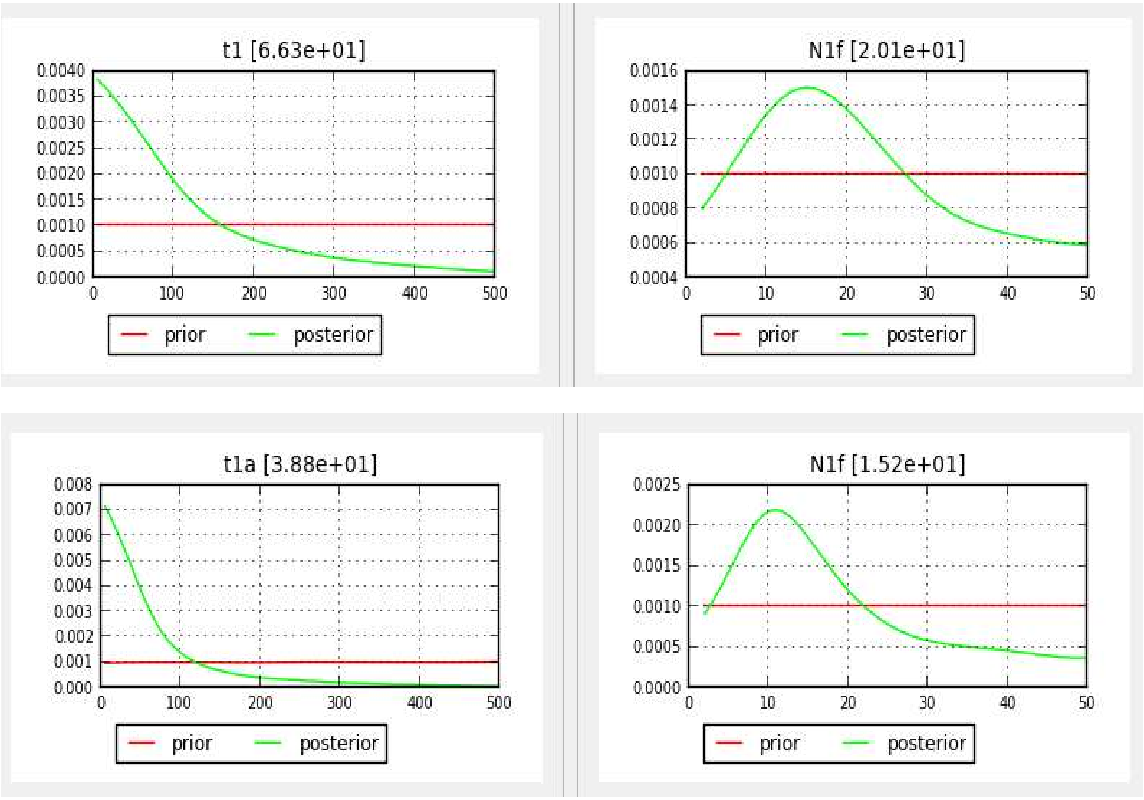
D. Model checking of final supported scenario: model fit PCA plot placing the location of the observed dataset (yellow circle) within the cloud of simulated posteriors (outlined circles).

	parameter	prior
N NRS adults across scenario	N1	u[10,10^4]
N NRS young across scenario	N2	u[10,10^4]
N DS across scenario	N3	u[10,10^4]
origin NRS from DS	t1a	u[5,500]
duration bottleneck	db	5
N during bottleneck	Nf1	u[2,50]
time of admixture NRS/DS	t2	u[5,500]
proportion admixture from NRS	r	0 - 1

Prior parameters for simulations
Times are in generations before time of sampling (t0)
u: uniform
db: duration of bottleneck (fixed at five generations)
r: varied between comparisons – see above

Figure SI 2.3 Inference of demographic parameters from posterior distributions of supported scenarios

A



A. Posterior distributions for origin of NRS from DS showing similarities between different model approaches

Upper panels: simple model of NRS from DS at time t1 with foundation bottleneck N1f

Lower panels: model with origin of NRS from DS at time t1a with foundation bottleneck N1f with subsequent admixture between NRS adults and DS to generate NRS young

Bottleneck duration fixed at 5 generations for both scenarios. Values above graphs are medians Times in generations are influenced by mutation model and overlapping generations, so cannot be taken as accurate estimate of actual parameter. However, modal value close to lowest value for priors, constrained by prior for bottleneck duration, is consistent with a recent (1999) origin of NRS

B

duration bn (generations)	N during bottleneck mode	(prior u [2,50]) q (0.050 - 0.950)
1	2.0	2.78 - 43.8
2	5.68	3.92 - 46.6
3	7.4	4.25 - 44.1
4	12.5	6.48 - 46.5
5	13.9	7.21 - 46.1

B. Posterior distributions for size of foundation bottleneck of varying duration

Scenarios in simple model of origin of NRS from DS with foundation bottleneck with different fixed values of db prior between 1 and 5. Posterior probability confidence intervals were overlapping for scenarios with the different values.

References SI.2 ABC modelling

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SI: Genotype data

HNR & DS age groups																
npops = 2																
nloci = 8																
	CA71		Vu4		Vb9		Vu57		CA3		CA11		Vb5b		Vb3	
pop = adult																
H1	114	124	163	177	154	158	140	140	165	165	180	202	220	246	204	204
H8	114	124	159	177	154	170	138	140	161	165	166	202	246	246	204	204
H9	124	124	165	177	154	154	140	140	161	165	166	180	220	246	204	226
H10	114	124	159	177	154	170	138	138	165	165	202	202	220	246	204	226
H12	114	124	159	177	158	170	138	140	165	165	202	202	246	246	204	226
H13	114	124	159	159	158	170	138	140	161	165	166	202	220	246	204	226
H19	114	124	163	177	154	158	140	140	165	165	180	202	220	246	204	204
H22	114	124	145	159	154	158	138	140	161	165	180	202	246	246	?	?
H26	114	124	159	177	158	170	138	140	165	165	166	180	220	220	204	226
H30	114	124	159	177	170	170	140	140	161	165	166	166	220	246	204	226
H2	114	124	163	177	154	170	138	140	165	165	202	202	220	246	204	204
H3	114	124	159	165	154	170	138	140	161	165	180	202	220	246	226	226
H4	114	124	159	165	154	154	138	140	161	165	166	202	246	246	204	226
H6	114	124	159	177	154	170	138	140	165	165	166	202	220	246	204	204
H7	114	124	159	165	154	170	138	140	161	165	180	202	220	246	208	226
H11	114	124	163	177	154	158	140	140	165	165	180	202	220	246	204	204
H14	114	124	159	165	154	170	140	140	165	165	166	180	220	246	204	226
H15	114	124	159	177	154	170	138	140	165	165	166	166	220	246	204	226
H18	114	124	177	177	158	158	140	140	161	165	166	180	220	246	208	226
H24	114	124	159	165	154	158	138	138	165	165	202	202	220	246	204	226
H25	114	124	159	177	154	154	138	140	161	165	166	202	220	220	204	226
pop = young																
nn35	114	124	159	177	154	170	138	140	161	165	166	180	246	246	204	226
nn36	114	124	165	177	154	158	138	140	165	165	166	202	220	246	204	204
nn37	114	124	159	177	154	170	140	140	165	165	166	180	246	246	204	226
nn38	114	124	159	165	154	170	140	140	165	165	166	166	246	246	204	226
nn39	114	124	163	177	154	154	138	140	161	165	166	202	246	246	204	204
H17	114	124	159	159	154	158	140	140	165	165	166	180	220	220	204	226
H20	124	124	163	177	170	170	138	140	161	161	180	202	220	246	204	226
H28	114	124	163	177	154	170	140	140	161	165	166	180	220	220	208	226
H5	114	124	159	177	158	170	140	140	161	165	166	202	220	246	204	226
H16	114	124	159	165	154	158	138	140	165	165	166	202	220	246	208	226
H21	114	124	159	177	158	170	138	140	165	165	202	202	246	246	204	226
H27	114	124	159	165	154	158	138	140	165	165	166	202	220	246	208	226
H29	114	124	159	177	158	170	138	140	161	165	202	202	220	220	?	?
H31	124	124	159	177	158	170	138	140	161	165	166	180	220	220	204	226
DS1	114	124	163	177	154	158	138	140	165	165	166	172	220	220	204	226
DS2	114	124	163	177	170	170	140	140	165	165	166	166	220	246	226	226