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NEXT ISSUE

First Breeding of the Red-necked Amazon (Amazona arausiaca)

ASA
BULLETIN

The purposes of the Society are the study of foreign and native birds to promote their conservation and protection; the dissemination of information on the care, breeding, and feeding of birds in captivity; the education of Society members and the public through publications, meetings, and available media; and the promotion and support of programs and institutions devoted to conservation. Front Cover: Spix's Macaw *(Cacatua alba)* Photo: Simon Degenhard. Inside cover: Red-necked Amazon *(Amazona arausiaca)* Photo: Simon Degenhard.

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President's Message

Greetings, fellow Aviculturists:

Long time.

Don't let the date fool you. As of this late publishing, the Spix's macaw (Cyanopsitta spixii) has been released into its ancestral homeland by Association for the Conservation of Threatened Parrots (ACTP). I highly recommend visiting their Facebook page for the latest information on the release.

This issue brings an aspect of the Spix's macaw program that might benefit other endangered birds: artificial insemination. Long utilized in the propagation of various mammals, the success in avian species is a promising tool for aviculturists working with endangered species.

On a solemn note, ASA recognizes the passing of past president Ivo Lazzeroni—his life is recounted by Steve Duncan. Gene Hall is also remembered in words and photos in this issue with an article by Dick Schroeder.

John Del Rio asks the question, "Why aviculture?" and also provides his "elevator" pitch which you may find useful.

There's so much more in this issue! Dive in and let us know what you would like to see in the future.

Stay safe and take care,

Carol Stanley, President, YOUR Avicultural Society of America

Officers & Staff

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Spix's Macaw (*Cyanopsitta spixii)* Photo: Simon Degenhard

A Novel Method for Semen Collection and Artificial Insemination in large parrots (Psittaciformes)

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The paper described a novel technique for semen collection in large psittacines (patent pending), a procedure which was not routinely possible before. For the first time, a large set of semen samples is now available for analysis as well as for artificial insemination. Semen samples of more than 100 psittacine taxa were collected and analysed; data demonstrate large differences in the spermatological parameters between families, indicating an ecological relationship with breeding behaviour (polygamous versus monogamous birds). Using semen samples for artificial insemination resulted in the production of offspring in various families, such as Macaws and Cockatoos, for the first time ever. The present technique represents a breakthrough in species conservation programs and will enable future research into the ecology and environmental factors influencing endangered species.

Many psittacine species are threatened by the destruction of their natural habitat or poaching. Therefore, captive breeding programs are important to preserve their genetic material and

to allow the later reintroduction of birds back to the wild. In some highly endangered species [e.g. Spix's Macaw (Cyanopsitta spixii)1; St. Vincent Amazon (Amazona guildingii)[2]], only a few individuals have survived in captivity. For captive breeding, birds are often forced-paired, resulting frequently in infertile eggs, which is similar to the case of male infertility. Artificial insemination would overcome such problems, which would also allow the inclusion of surplus males or females in the breeding population3. Artificial insemination can also be used to increase the representation of genetically valuable specimens within a population. Semen collection and the subsequent evaluation of sperm can also provide valuable information about male fertility.

In birds of prey[4], turkeys[5], cranes6 or pigeons[7], semen collection and subsequent artificial insemination is frequently used. In contrast, semen collection in larger psittacine species has only been reported anecdotally8–10, and has never been successful in a larger set of different species or individuals. Consequently, successful artificial insemination has only been reported for a few small psittacine species or single cases, but never for larger endangered parrots[9],11–13. In this report we describe a novel technique of semen collection which was successful in 109 different psittacine species. The collected semen was employed for quality evaluation and artificial insemination which was successful in several species. The results of this study represent a breakthrough in species conservation projects of large psittacines and allow further research on spermatological parameters and their link to ecological niches of the species.

Results

Semen collection. Applying the novel method in 243 different males (see Table 1), 230 out of 344 attempts (66.9%) at semen collection were successful. In 72 cases, the males were classified as Group 1 (see Methods), of which 67 were successful (593.1%). Of 272 attempts from Group 2 (see Methods), 163 attempts (559.9%) were successful.

Semen evaluation. A complete semen examination was only possible in 81 samples due to the limited volume or the need for artificial insemination of the samples. The results of semen analysis differed between psittacines families, subfamilies or tribes. Volumes and sperm concentrations of Eclectus/ Tanygnathus and Cockatoos were the highest, whereas the Amazons and Macaws resulted in lower values (see Tab. 2). Birds from the same family, subfamily, tribe or species exhibited comparable semen quality.

Spix's Macaw (*Cyanopsitta spixii)* Photo: Simon Degenhard

Table 1 | List of parrots in which semen collection was attempted. Group 1 represents paired and sexually active males in which successful semen collection was expected, as they have previously produced fertile clutches. Group 2 represents single housed or sexually inactive males or birds which had previously produced infertile clutches, so successful semen collection was not expected.

Species	Attempts of Collection	Successful Semen Collection		
	Total	Total	Group 1	Group 2
Cacatua leadbeateri	1	1	0	1
Cacatua haematuropygia	11	11	1	10
Cacatua sulphurea sulphurea	1	1	0	1
Cacatua sulphurea citrinocristata	1	1	0	1
Cacatua sulphurea abbotti	1	1	0	1
Cacatua alba	1	0	0	1
Cacatua galerita galerita	4	4	0	4
Cacatua pastinator	1	1	0	1
Cacatua tenuirostris	2	1	0	2
Cacatua ducorpsii	1	1	0	1
Cacatua sanguinea sanguinea	1	1	0	1
Cacatua goffini	2	1	0	2
Cacatua ducorpsii	1	1	1	0
Cacatua opthalmica	1	1	0	1
Cacatua moluccensis	1	1	1	0
Calyptorhynchus magnificus banksii		2	2	0 2
Calyptorhynchus funereus funereus		1	1	0 1
Calyptorhynchus funereus latirostris		5	5	4 1
Callocephalon fimbriatum	4	4	0	4
Eolophus roseicapilla	1	1	0	1
Nymphicus hollandicus	1	1	1	0
Probosciger aterimus aterimus	2	2	0	2
Probosciger aterimus goliath	1	1	0	1
Cockatoos total:	47	44		Success rate: 93.6%
Anodorhynchus hyacinthinus	3	3	3	0
Anodorhynchus leari	2	1	1	1
Ara ambiguus	3	2	1	2
Ara chloropterus	7	5	4	3
Ara glaucogularis	9	4	3	6
Ara militaris militaris	1	1	0	1
Ara nobilis cumanensis	2	1	0	2
Ara severus	1	0	0	1
Dipsittaca nobilis nobilis	1	0	0	1
Primolius auricollis	1	0	0	1
Ara ararauna	2	0	0	2
Ara macao	1	0	0	1
Ara rubrogenys	1	0	0	1
Cyanopsitta spixii	4	3	0	4
Primolius couloni	6	4	2	4
Macaws total:	**44**	**24**		**Success rate: 54.5%**

Table 1 | continue

Amazona guildingii	3	3	1	2	
Amazona pretrei	32	24	2	30	
Amazona vinacea	3	2	1	2	
Amazona xantholora	6	4	0	6	
Amazona rhodocorytha	2	1	0	2	
Amazona brasiliensis	1	1	1	0	
Amazona albifrons nana	1	1	0	1	
Amazona tucumana	4	4	1	3	
Amazona amazonica	1	1	0	1	
Amazona finschii	2	2	0	2	
Amazona viridigenalis	3	3	2	1	
Amazona barbadensis	1	1	0	1	
Amazona ochrocephala oratrix	1	0	0	1	
Amazona ochrocephala xatholaema		1	0	1	
Amazona auropaliata	1	0	0	1	
Amazona mercenaria	1	0	0	1	
Amazona leucocephala	1	0	0	1	
Amazona festiva festiva	1	0	0	1	
Amazona dufresniana	3	1	0	3	
Amazona farinosa guatemalae	1	1	0	1	
Amazona farinosa virenticeps	1	1	1	0	
Amazona festiva bodini	8	3	3	5	
Amazona autumnalis lilacina	7	5	1	6	
Amazona autumnalis autumnalis	9	7	1	8	
Amazona ventralis	1	1	1	0	
Alipiopsitta xanthops	2	2	0	2	
Amazons total:	**97**	**68**		**Success rate: 68.7%**	
Eos histrio	2	2	1	1	
Eos squamata squamata	1	0	0	1	
Eos squamata riciniata	1	1	0	1	
Chalcopsitta sintilata	2	1	0	2	
Chalcopsitta atra	1	1	0	1	
Charmosyna papou	2	2	0	2	
Charmosyna pulchella	1	1	0	1	
Chalcopsitta cardinalis	1	0	0	1	
Glossopsitta concinna	1	1	1	0	
Lorius lory	2	1	0	2	
Lorius garrulus flavopalliatus	1	1	0	1	
Lorius chlorocercus	1	1	1	0	
Loriculus philippensis	1	0	0	1	
Oreopsittacus arfaki	2	0	0	2	
Trichoglossus haematodus deplanchii		1	1	1	0
Trichoglossus haematodus capistratus		3	3	0	3
Trichoglossus euteles	1	1	0	1	
Trichoglossus haematodus rosenbergii		2	0	0	2
Pseudeos fuscata	2	1	1	1	
Vini australis	1	1	0	1	
Lorys total:	**29**	**19**		**Success rate: 65.5%**	

Table 1 | continue

Species	Attempts of Collection Total	Successful Semen Collection Total	Group 1	Group 2
Eclectus roratus roratus	1	1	0	1
Eclectus roratus vosmaeri	1	1	0	1
Eclectus roratus salomonensis	6	6	2	4
Eclectus roratus polychloros	13	12	13	0
Eclectus roratus aruensis	1	1	1	0
Tanygnathus lucionensis	4	4	1	3
Tanygnathus sumatranus	3	3	1	2
Tanygnathus/Eclectus total:	**29**	**28**	**Success rate: 96.6%**	
Agapornis lilianae	1	1	0	1
Agapornis taranta	2	1	0	2
Agapornis roseicollis	1	0	0	1
Alisterus amboinensis amboinensis	1	1	0	1
Alisterus c. moszkowski	1	1	0	1
Aratinga pertinax pertinax	2	1	1	1
Aratinga pertinax surinama	1	1	1	0
Aratinga solstitialis	1	1	0	1
Aratinga cactorum	1	1	0	1
Aratinga wagleri minor	3	1	0	3
Aratinga rubritorquis	1	0	0	1
Aratinga erythrogenys	1	0	0	1
Aratinga holochlora	1	0	0	1
Aratinga acuticaudata	2	0	1	1
Aratinga leucopthalmus	1	0	0	1
Aratinga wagleri	1	0	0	1
Barnardius zonarius semitorquatus	1	1	0	1
Brotogeris chrysopterus tuipara	1	1	0	1
Brotogeris cyonoptera gustavi	1	1	1	0
Brotogeris cyanoptera	1	0	0	1
Caracopsis nigra	1	0	0	1
Cyanoliseus patagonus	1	1	0	1
Deroptyrus accipitrinus	2	1	0	2
Forpus conspicitatus	1	0	0	1
Guaruba guarouba	2	1	0	2
Nestor notabilis	2	2	0	2
Psittacula chaltorpae	3	2	0	3
Psittacula derbiana	1	1	0	1
Psittacula himalayana	1	1	0	1
Psittacula krameri krameri	1	1	0	1
Psittacula eupatria siamensis	1	0	0	1
Psittacula alexandrii	2	0	0	2
Psittacus erithacus erithacus	5	0	0	5
Psittacus timneh	9	4	4	5

Table 1 | continue

Species	Attempts of Collection Total Total	Successful Semen Collection Total Total	Group 1 Group 1	Group 2 Group 2
Pionopsitta pileata	7	5	0	7
Pionus maximiliani	3	0	0	3
Pionus menstruus	1	0	0	1
Pionitis leucogaster leucogaster	2	0	0	2
Platycercus elegans	1	0	0	1
Platycercus adelaide	1	1	0	1
Poicephalus robustus fuscicollis	1	1	0	1
Poicephalus meyeri	1	1	1	0
Poicephalus senegalus mesotypus	1	1	0	1
Poicephalus rueppeli	2	1	0	2
Poicephalus rufiventris	3	0	0	3
Poicephalus senegalus	1	0	0	1
Poicephalus guielmi	1	0	0	1
Psephotus h. haematogaster	1	1	0	1
Psittrichas fulgidus	1	1	0	1
Purpureicephalus spurius	1	0	1	0
Pyrrhura cruentata	2	1	0	2
Pyrrhura picta roseifrons	1	1	0	1
Pyrrhura leucotis emma	1	1	0	1
Pyrrhura hoffmanni gaudens	2	2	0	2
Pyrrhura melanura souancei	2	2	1	1
Pyrrhura rupicola rupicola	1	1	0	1
Pyrrhura perlata perlata	1	1	0	1
Pyrrhura molinae restricta	1	0	1	0
Rhynchopsitta pachyrhyncha	1	0	0	1
Triclaria malachitacea	1	0	0	1
Others total:	**98**	**47**	**Success rate: 48.0%**	
(total species: 151)	(total n 5 288)	(total n 5 230)	(total success rate 79.9%)	

Artificial insemination. In total, 64 inseminations were performed, which included repeated inseminations of the same female (12 cases). 15 samples were inseminated directly after intracapillary examination without a detailed analysis (see Table 3). After artificial inse- mination, 25 fertile eggs were obtained out of the 36 eggs laid (69.4%) by eleven females (see Table 4). Altogether, in nine out of 25 species, fertilised eggs were produced by artificial insemination (see Tab. 4).

A successful fertilisation by artificial insemination was confirmed by DNA fingerprinting in all Eclectus and one of the two Cacatua haematuropygia cases investigated. The Pionopsitta pileata juveniles had derived from natural mating of the females with their respective partners. In the other cases, both males (partner male and semen donor) were too closely related to achieve an unambiguous paternity test (see Table 4). However, as clutches prior to and after the study of those pairs remained infertile it seems likely that the technique had been successful even in those unresolved cases.

Discussion

For the first time, a technique for semen collection in psittacines was successful in a larger number of different species. This allowed the comparison of semen parameters between species as well as assisted reproduction techniques in species conservation programs. Using this technique, semen collection was frequently more successful in some parrot groups or families than in others (see Table 1). This could be related to the corresponding breeding seasons because the study was only performed during three months in the spring. Psittacines have a relatively short time of sexual activity14. As semen volume and sperm concentrations are significantly affected by the season15, the timing of successful semen collection is extremely important. It is unlikely that the differing success rates are related to the method itself, as it was generally successful in all families. Individual failure of the technique might also be explained in cases where males copulated with their partners immediately prior to the

semen collection procedure, as this has been previously described as a potential reason for failure in semen collection16. However, this needs to be evaluated in further studies. In general, this novel technique did not result in any case of macroscopic physical irritations or negative changes of behaviour. In many breeding pairs, especially in Eclectus spp. and Tanygna- thus spp., it was possible to collect high concentration semen samples from nearly all of the males, although most clutches of those species were infertile. Certain factors besides male infertility (e.g. housing system) may be responsible for this problem. Eclectus parrots (Eclectus roratus) have a particular mating system compared to other parrots17, in that a female is naturally paired with several

males. In addition, we assume that polygamy is the reason for the high sperm count in those species, because the semen of different males has to compete for the same follicle. Similar observations have been prev- iously described, demonstrating that testicular size is significantly larger in species that breed in colonies compared to species that breed solitarily18. However, several semen samples were not investigated in detail, as the volume was too low or the sample was used for artificial insemination. Future studies should therefore focus on a more detailed semen analysis in single species to establish reference para- meters, which would also allow a better comparison between species. Additionally, spermatozoa survival studies using this technique should be performed. Also, the possibility that semen quality might be reduced due to the electrical technique cannot be excluded. However, as no other technique for semen collection in large psittacines is routinely available to date, this cannot be investigated in the species used here at present. Using the collected semen for artificial insemination, fertilised eggs were obtained, even when samples of low sperm concentration were transferred: for example the Green winged Macaw (Ara chlor- opterus) was inseminated with 19.2 ml semen (concentration of 9000 sperm/ml and a total sperm number of 172,800) prior to first oviposition and 18.2 ml semen [containing sporadic sperm cells (1)] after the first egg. In this case, the second of the two eggs was fertile. The number of sperm cells was much lower than normally required for successful insemination (i.e., 20,000,000 spermatozoa) in free-ranging birds19 or chickens20. This should be kept in mind when working in a species conservation program with a limited number of available males of reduced semen quality. Obviously they still could be genetically included in the population.

Importantly, divided semen samples (e.g. Red-sided Eclectus Parrot (Eclectus roratus polychlorus)) resulted in offspring from different females. In particular, one sample of 17.1 ml (1,590,000 sperm/ml) formed three aliquots (two 5 ml and one 6.5 ml), fertilising the eggs of three different females. This is a very important result, as this will allow the representation of genetically valuable males in an endangered population to be increased. However, it must be taken into account that egg-infertility after artificial insemination might be caused by poor semen quality, as well as by the time of insemination. The fertility period of the hen and each egg to be laid, as well as the average spermatozoon survival time in the oviduct, vary greatly between species21 and are unknown in the psittacine species used in this study. Therefore, more experience must be collected and further studies performed to investigate the optimal time and frequency of insemination to

Table 2 | Semen parameters of selected families, subfamilies or tribes

Group	volume (ml)	sperms/ml
Cockatoos (n 5 21)	12.4	427704
	(1.8–54)	(850–4 500 000)
Macaws (n 5 6)	7.4	70068
	(3–19.5)	(27 500–150 000)
Amazons (n 5 11)	8.5	77532
	(2.8–18.4)	(8750–231 000)
Eclectus ssp./Tanygnathus spp. (n 5 26)	11.4	3781285
	(1–29)	(187 000–16 000 000)
Loris (n 5 12)	6.3	1534965
	(1.3–12.6)	(29583–5050000)
Others (n 5 21)	7.1	1317526
	(1.5–29.8)	(26500–10 450 000)

achieve the best fertility results. Therefore, the values provided for the semen samples (see Table 3) which did not lead to a fertile egg are just for information purposes. The quality of those samples cannot be contemplated the reason for infertility, as many other reasons must also be considered (see above). Most importantly, the values of those semen samples which lead to a fertile egg should be used as a guideline until more data are available. However, it is worth noting that semen collected with the novel electrical-based technique is able to fertilise eggs.

Overall, although our novel approach was highly successful, it must be kept in mind that semen samples are able to transmit different viral22,23 and bacterial24,25 diseases. Therefore, it is very import- ant to examine the health status of a semen donor prior to semen collection.

It must be noted that 27 out of 64 artificial inseminations did not lead to oviposition. It can be speculated that the insemination procedure had interrupted breeding behaviour. In these cases, the females had been selected at the beginning of the study according to their expected breeding condition. After gaining some experience, we selected females for insemination only after breeding behaviour occurred or after a first egg was laid; as a consequence, clutches were always completed in these cases. However, it cannot be completely excluded that artificial insemination does not interfere with breeding activity.

While it has not been possible to obtain repeated semen samples from larger psittacine species in the

total sperm number	L-D	motility	Forward motility
5183354	90.3	77.9	71.5
(10540–69300000)	(71–6.5)	(40–85)	(30–80)
435750	87.1	75.8	70
(41300–806664)	(81–2.5)	(70–85)	(60–80)
675050	87.9	80	75
(101050–2263800)	(83.5–91)	(all 80)	(all 75)
35828804	94.6	87.3	82.3
(550000–157200000)	(87–99)	(60–95)	(55–90)
9806674	95.9	86.8	81.4
(82832–41265000)	(92–99.5)	(70–95)	(60–95)
5318745	89	80.7	75
(53000–33440000)	(55–99)	(55–95)	(50–90)

past, the newly developed technique allows semen collection and evaluation in Psittaciformes for the first time. The novel technique is based on electro-stimulation, which has previously been described in waterfowl26,27. The significant alterations in probe design and electrode placements enabled the success of this technique in psittacine birds. However, it must be kept in mind that electrical stimulation for semen collection might affect the semen quality and only the proof to enable fertilisation described the success of a collection method. This was the case in the present study. This novel technique enables the precise and non- invasive assessment of the fertility of males as a prerequisite for successful artificial insemination. In the past, a biopsy of the testes was often the main method for the investigation of male fertility28. Furthermore, it initially enables the development of semen reference values and research about cryopreservation in large psittacines, especially in highly endangered species. However, bearing in mind that semen was obtained from Spix's macaws (Cyanopsitta spixii), this method is a major breakthrough in species conservation projects and might be a valuable method to assist the survival of this species. It allows the inclusion of surplus males into a breeding collection, but also the inclusion of surplus females which are present in many species conservation breeding programs (e.g. the Spix's Macaw). Such females can be paired with a sterilised partner of a closely related species being artificially inseminated with semen of her own species29.

To best of our knowledge, this study resulted in the world's first

Table 3 | Semen parameters in samples used for artificial insemination: Volumes, sperm-concentrations and total sperm cells used for AI as well as fertility of the obtained clutches. Only clutches with successful or likely successful artificial insemination are listed. Each row represents one female and each semen data one artificial insemination

Species	AI before first oviposition	AI after first oviposition	AI after second oviposition	Total eggs/potentially fertilised by AI/fertilised
Cacatua haematuropygia	**1.)** 5.3 µl (volume) +++ (concentration) **2.).** 5.1 µl +++			2/2/2
Ara chloroptera	19.2 µl 9000/µl 172800 (total sperms)	18.2 µl +		2/2/1 Second egg fertilised
Amazona pretrei		8.3 µl ++	10.3 µl +	4/3/2 Second and third egg fertilised
Amazona pretrei		2 µl +		5/3/0 AI 1 day after first egg, second egg not potentially fertilised with AI
Amazona xantholora		6 µl +++	8 µl 25000/µl 200000	4/3/3
Amazona finschii		7 µl 140000/µl 980000		4/3/1 Second egg fertilised
Tanygnathus lucionensis	3.1 µl 16000000/µl 49600000	3.8 µl 14 840000/µl 56392000		3/3/2 Second and third egg fertile
Eclectus roratus polychlorus	**1.)** 7 µl 2725000/µl 19075000 **2.)** 6.5 µl 1590000/µl 10335000			2/2/2
Eclectus roratus polychlorus	5 µl 2725000/µl 13625000	5 µl 1590000/µl 7950000		3/3/2 Second and third egg fertile
Eclectus roratus polychlorus	**1.)** 10 µl 6000000/µl 60000000 **2.)** 5 µl 1590000/µl 7950000			2/2/2

Second to fourth column: volumes and concentrations used for AI (1. = first insemination before first egg; 2. = second insemination before first egg. If semen was only examined within the capillary, the concentration is graded like described in the text (sporadic (+), low (++), middle (+++), high (++++) and extremely high (+++++)). Fifth column: 4/3/2 = In a clutch with a total of 4 eggs, 3 eggs were laid after AI (potentially fertilised) and 2 were effectively fertilised.

Table 4 | Success after artificial insemination

Species	No. eggs after AI	Fertile eggs	Fertility rate (%)	Genetic tests
Cacatua haematuropygia	2	2	100	1 juvenile result of AI
Calyptorhynchus funereus latirostris	2	2	100	Semen of own partner was used - no test
Ara chloroptera	2	1	50	Two females were partners – no test necessary
Amazona pretrei	6	2	33.3	No result: partner male and semen donor brothers
Amazona xantholora	3	3	100	No result: Semen donor son of inseminated mother
Amazona finschii	3	1	33.3	No result: partner male and semen donor brothers
Amazona guildingii	2	0	0	
Amazona viridigenalis	1	1	100	Not possible due to very early embryonic death
Tanygnathus lucionensis	3	2	66.3	No test, eggs were eaten by parents
Eclectus roratus polychlorus	7	6	85.7	Juveniles result of AI
Pionopsitta pileata	5	5	100	One egg eaten, partner male is the father of all juveniles
total	**36**	**25**	**69.4**	

AI = Artificial insemination.

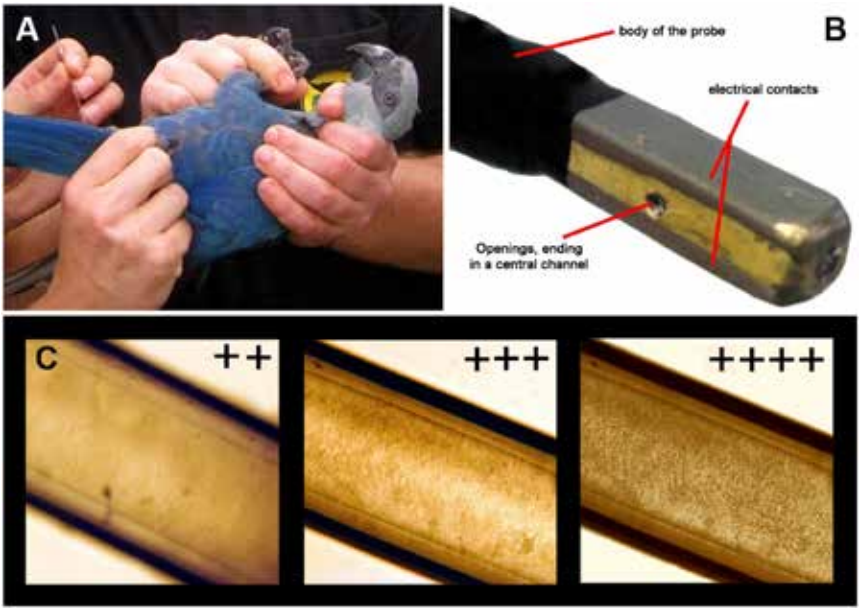

Figure 1 | Semen collection and artificial insemination. (A) Fixation of the male during semen collection process in a Spix's macaw (Cyanopsitta spixii). (B) The head of the probe used for the semen collection. A micro-glass capillary has to be inserted from behind. (C) Micro capillary filled with semen classified 11 (low), 111 (middle) and 1111 (high) in concentration (4003 magnification).

assisted reproduction macaw (Ara chloropterus) as well as Pionopsitta pileata, Cacatua haematuropygia and species of Psittaculinae.

Methods

Birds. In this study, 280 (243 males, 37 females) psittacines of 151 species and subspecies of different families were included (see Table 1). They were housed in aviaries as pairs, groups or single birds, receiving feed (seed mixture, fruit and vegetables) and fresh drinking water twice a day. Females which had repeatedly produced infertile eggs were selected for artificial insemination, and there was also a pure female pair of Green winged Macaws (Ara chloroptera). Males included for semen collection were divided into two groups: Group 1: Paired and sexually active males with frequently fertile clutches in the past; Group 2: Single males, sexually inactive males, or those with problems in reproduction in the past.

Semen collection method. The birds were caught directly prior to semen collection and immediately placed on their back. No sedation or anaesthesia was applied.
Assisted by a second person, the cloaca was visualised (see Fig. 1A). The semen collection was carried out by an electrical stimulation

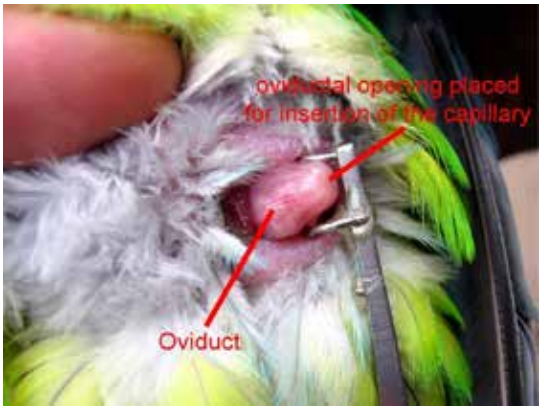

Figure 2 | Visualisation of the oviduct opening in Pionopsitta pileata.

procedure with a newly developed bipolar probe (Patent pending). The probe consists of non-conducting plastic, where two electrical contacts are placed at the sides. Two small openings in the head of the probe end in a central channel (see Fig. 1B), through which a glass micro- capillary can be inserted from behind. The ejaculate sequesters automatically in the capillary after semen disposal. The size of the probe correlated with the size of the respective bird. First, the probe was introduced into the cloaca until both electrodes were completely inside; this ensured contact with the cloacal wall. Importantly, the probe should not be inserted as far as the rectum and should be specifically located with the majority in the urodeum. A slight electric current (between 1 and 14 V) was applied in short 1–2 seconds intervals (with breaks between 2 and 5 sec, slightly raising the voltage) until contractions of the cloaca and the muscles of the tail were observed and ejaculations had occurred. In most individuals, 6 V was ideal, with 5–8 intervals necessary to reach ejaculations, depending on the individual. The amperage depends physically on the electric resistance of each individual and can therefore not be provided as a general rule. The complete procedure of semen collection was usually completed within 3–5 minutes. In cases where 10 intervals within 5 minutes did not lead to ejaculation, the attempts were counted as failures. After the removal of the capillary from the probe, the semen was evaluated macroscopically.

Semen evaluation. In the capillary, sperm-concentration, motility and contamination of the semen sample were evaluated. The sperm concentration was graded as sporadic (1), low (11), middle (111), high (1111) and extremely high (11111) (see Fig. 1C) using a microscopic 1003 magnification. Motility was graded as motile and immobile. The contaminations were assessed macroscopically and graded as low (1), middle (11) and high (111). Afterwards, the semen samples were used for artificial insemination and/or for further evaluation. In many samples

used for artificial insemination, a more detailed examination was not possible due to the limited amount available. A more detailed examination would have required the complete amount, not leaving any for the insemination procedure. In the semen samples investigated (.5 ml), the volume and pH of ejaculates, as well as motility, vitality, morphology, concentration and total number of spermatozoa were investigated11,12,30. Volume was measured via scaled capillaries and pH using commercial indicator paper. Motility was estimated within 5 fields of view on a pre- warmed slide (microscopic magnification 1003). Sperm concentration and total sperm count was assessed using a Neubauer's counting chamber. Eosin B (2%) was used as live/dead stain for vitality analysis and for assessment of sperm morphology as well11,30.

Artificial insemination. Insemination was performed in different species (Table 3), using semen without contamination and with motile spermatozoa 1–2 days prior to the expected oviposition or directly (within 2 hours) after the first egg was laid.

Oviposition was expected due to observed breeding behaviour or typical abdominal extension. If possible, females were inseminated with semen from males other than their partners. The females were turned on their back and the oviduct opening was everted within the cloaca, followed by semen deposition into the oviduct (see Fig. 2). For this, the semen was pushed directly from the capillary into the oviduct by a plunger as previously described11. After 8–10 days of incubation in eggs which were laid after insemination, fertilisation was assessed by candling. Eggs with visual embryonic development were classified as fertile.

Assessment of parentage by DNA fingerprinting. Dead in shell embryos or hatched chicks were subjected to a paternity test by multilocus DNA fingerprinting, to exclude the female's partner as a father and to prove successful insemination.

Total DNA was isolated from 100 ml of blood using standard proteinase K (Merck, Darmstadt) and phenol/ chloroform procedures31. Multilocus DNA fingerprinting was carried out to investigate the genetic relationships among and between the sampled birds following standard protocols32. The evaluation of the DNA fingerprints followed some fundamental rules33 by trying to assign all bands of the nestlings to the putative parents. The informative (i.e. polymorphic) bands of the DNA fingerprint (usually between 10 and 20) were visually scored into a data matrix as either absent ("0") or present ("1"), which was then used to calculate the band-sharing coefficients (BSC) as BSC 5 C 3 2/(A 1 B), with C being the number of shared bands, and A and B the number of bands in the profiles of individual A and B, respectively34.

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Author contributions
M.L. and D.N. developed the technique, M.L. planned the study, wrote the manuscript and participated in the field work, D.N. contributed to the study design, the manuscript preparation and performed the field work, H.M. and M.R. assisted in the field work and the selection of the birds, M.W. contributed to the manuscript and performed all laboratory tests for the paternity analysis.

Additional information

Additional Information: Herewith the authors declare, that the study (manuscript no: SREP-12-03370A) was done in accordance with the national laws and that the method used in this manuscript was approved for use in birds by the Regierungspra¨sidium (Regional council) Giessen, Germany, with the permission number V54-19C 20-15 (1) GI18/9 Nr. 77/ 2009.
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- Animal loving public generally unaware of activities to end their having pets, eating meat, medical research, entertainment, education, etc.
- YOU can help: Take ACTION when asked. Watch for proposed BAD laws.
- Call your senator or representative when asked - always be respectful & polite
- Respond only with truth
- Join National bird organizations: AFA, ASA, OPA, SPBE and DONATE to legislative causes when asked
- INFORM OTHER BIRD AND ANIMAL LOVERS about this

- Continuously lobbying the end of all animal use: Food, entertainment, pets, conservation, medical research
- Have warchests of money collected based on lies from unsuspecting animal lovers
- Target retirement savings of unsuspecting seniors
- Most income goes to marketing and legal fees
- Use misleading marketing practices to steal dollars from unsuspecting pet lovers
- Hypocrites: Personally use medical advancements developed through research
- Make used car salespeople look like saints

Spix's Macaw Reintroduction Becomes Reality

Simon Degenhard

Today, the 7th of June 2019, the strongest message yet, that the Spix's Macaw will be returned to wild, was delivered to the world.

Representatives from ACTP, Pairi Daiza Foundation and Parrots International met with the Government of Brazil to sign the most significant of contracts; the contract that guarantees the return of this small blue macaw to its home, in the Brazilian Caatinga.

There have been many reports in world media making various claims about the newly elected Brazilian Government's apparent lack of interest in the protection of the environment and the endangered species that call Brazil home. Today the Government has shown that they are fully committed to the Spix's Macaw De-Extinction Project; making this conservation program a main priority.

In attendance, were Sra. Tereza Cristina - Minister of Agriculture and Vice Minister of Environment, Mr Homero de Giorge Cerqueira - President of ICMbio, Sra. Ana Maria Pellini – Executive Secretariat of the Ministry of Environment of Brazil, Sr. Eduardo Serra Negra Camerini - Secretariat of Biodiversity of the Ministry of Brazil, Sr. Roberto Castelo Branco Coelho de Souza - Secretariat of International Relations of the Ministry of Environment of Brazil, Martin Guth - President ACTP (German), Tim Bouts - Pairi Daiza Foundation (Belgium), Mark Stafford - Parrots International (USA) and Edson Gontijo - Facenda Cachoeira (Brazil), all sharing the same dream, to put everything into place to see the Spix's Macaw flying free in the wilds of the Brazilian Caatinga once again!

The 7th of June 2019 will be remembered as the day that the agreement to bring the Spix's Macaw back to the wild was confirmed.

The first 50 Spix's Macaws will arrive in Brazil at the completed Release Facility by the end of 2019. With a planned first release to take place soon after.

Link to announcement on Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) website:

http://www.icmbio.gov.br/portal/ultimas-noticias/20-geral/10357-acordo-garante-repatriacao-de-50-ararinhas-azuis

Spix's Macaw (*Cyanopsitta spixii)* Photo: Simon Degenhard

Oil painting by Australian wildlife artist Rachel Lewis
https://www.rachel-lewis.com.au/

Past President, Ivo Lazeroni
December 5, 1917 to March 6, 2022

Sheldon Dingle, left and Ivo Lazzeroni at an Avicultural Society of America Conference hosted by Wildlife World Zoo in Arizona. Photo: Carol Stanley

Ivo Lazzeroni passed away on March 6, 2022, at 104 years of age. Pictured here at a spry 90 years of age on the right at ASA's 2008 Conference in Phoenix, Arizona, he's standing next to Sheldon Dingle on the left, both very prominent ASA members who have passed on. Born December 5, 1917, for many years, he was the only member of ASA that was older than ASA, but he never looked it. He joined ASA in 1945 making him the longest continuous member as well. Ivo served on the Board of Directors of ASA for many years. He was extremely devoted to the organization. In addition to aviculture, he was also an enthusiastic model train hobbyist. He got the most out of his ASA Life Membership and the most out of life as well. An amazing and kind man, Ivo earned his wings long before March 6, 2022. Soar high, Lazzeroni!

Steve Duncan

Photos next page top: Ivo receives the Avicultural Achievement Award from Steve Duncan. Inset: Ivo and his granddaughter. Bottom: left to right; Daniel Shearing, Sheldon Dingle, Ivo Lazerroni.

Remembering Gene Hall

Dick Schroeder

Gene Hall June 12, 1928 - August 29, 2021

When I first got into birds, in the early 70's, living just north of LAX, there were not a whole lot of bird stores around, best known was Bates and Busenbarks' Palos Verdes Bird Farm. Sure, you could buy zebra finches and budgies at some department stores and dime stores, but no real choices.

Gene Hall had the Fortune Glen Aviaries, way down in San Marcos and he would run a small ad weekly in the LA Times advertising all sorts of nifty sounding birds. This was

before the I-5 freeway existed in that part of the state, so it was a lengthy drive from the LAX area. One weekend we decided to drive down and check it out.

It was wonderful! All sorts of exotic finches and parrots. I had just finished a new, planted, finch aviary and needed some inhabitants. This was the place! We bought 5 or 6 pairs of birds and made the long drive back home, arriving in the late afternoon. I hurried to get the birds out into their new home before dark.

Bad idea!! Next morning nearly half were dead on the ground. I couldn't believe it. What happened?? I went in and called Gene to tell him that the birds I'd just were dying, what could be the problem? He asked when did I put them in the flight? I replied, just as soon as I got home. They all looked fine then, I don't understand what the problem was. He explained to me that the birds needed time to settle in, find places to roost, and be ready for the night. I didn't provide them with enough time and should have kept them in their boxes and put them out first thing in the morning.

I had no clue, I just killed a bunch of birds that I was hoping to breed. Gene was a very kind soul. He told me to collect up the dead birds and bring them back to San Marcos and he replaced every single bird without charging me a dime.

Over the years I purchased many birds from Gene, but I learned two lessons that day. First, don't put birds out just before dark, and one huge lesson in customer service. By replacing the birds that I was responsible for killing, he had a customer for a very long time.

Liz Ferrell provided a home for Gene during the last years of his life. Below are excerpts from an email sent by Liz to Gene's email friends followed by photos of Gene throughout the years.

Liz email excerpts

My African grey, Sparky Bird, knows Gene by name and calls him "Gene, Gene, wanna come out." I take him to Gene and Gene would get on the floor and they would wrestle, tear up old phone books and then Gene would put him on his walker and take him to the front door and they would talk about the people walking by. Then walk through the house discussing all that they see talking of many things, of shoes, and ships and sealing wax, of cabbages and the King, our Lord.

I brought my grey up to see Gene, he couldn't get on the floor today, and he couldn't take him for his ride on the walker, so he climbed up on the bed, went to Gene's chest and still nothing. My grey finally put his head under Gene's bearded chin and said "I love you" Gene slowly brought up one hand, hugged my grey and with all that he had left, "I love you more" his hand slowly fell to his side. I took my grey and tried to play on the floor but he wasn't going to have that with Gene right there. I put him on the walker and tried to talk of what they did but he called for Gene. I brought him back to Gene and took Gene's hand and waved by, and my grey said his usual bye bye bye.


EUREKA

"Aviculture Elevation"

John Del Rio

Q: Why Aviculture?

A: Why not?

Like so many avocations in our society and culture today, Aviculture is being denigrated, hollowed out, and redefined by non-stakeholders and enemies. As Aviculturists, we find ourselves having to defend our very existence. Often, our rebuttals are not effective because we fall into the trap our enemies have set, which is: The False Premise.

This trap sets forth the premise that we, as Aviculturists, as humans, must be able to justify our desire and ability to keep birds. That somehow these detractors have a right to tell us what, where, when, and how we can enjoy our birds. They believe they have a moral superiority and the obligation to force that opinion on us. Our mistake is in accepting this false premise at all.

Destroy that premise! Give it zero heed. Zero oxygen. Zero attention, which is exactly what they want.

Instead, Aviculture should proudly stand on its own, both as individuals and collectively. We do not have to justify our love of birds. We do have to have a solid pitch for why we enjoy our birds. We do have to be able to present Aviculture in a positive manner.

Here is my "Elevator Pitch" for Aviculture:

Aviculture is a fantastic means of enjoying the spectacular variety of birds from around the world. Aviculture is essentially an art that combines science, biology, animal husbandry, massive amounts of love, and endless dedication.

The results of Aviculture are a greater understanding of birds, the sharing of information about their best care and reproduction, and the encouragement of others to take a serious interest in ecology and wildlife, with a focus on building a greater human-animal bond.

Aviculture is life well-spent.

-John Del Rio 3/14/22

Press release - 14 July 2021
The IUCN SSC Asian Songbird Trade Specialist Group:
A Brief Report On Its First Four Years: 2017-2020

The IUCN SSC Asian Songbird Trade Specialist Group (ASTSG) published a brief report outlining its work scope and achievements since its inception in 2017 and future plans for the tackling of the Asian songbird crisis. The report provides updates on the songbird conservation efforts led by the ASTSG's members between 2017 and 2020, under the group's five main themes.

Field-based surveys conducted informed the ASTSG's priority conservation actions and identified further research areas. The findings also contributed to the changes in several species conservation statuses. Among them are Straw-headed Bulbul (Pycnonotus zeylanicus), Sumatran Laughingthrush (Garrulax bicolor), Rufous-fronted Laughingthrush (Garrulax rufifrons) and Javan Pied Starling (Gracupica jalla).

Genetic research helped to identify new genomically distinctive populations of songbirds affected by the trade, notably Javan Jungle Flycatcher (Cyornis banyumas), Simeulue Hill Myna (Gracula religiosa miotera), Sangkar White-eye (Zosterops melanurus), Javan Pied Starling (Gracupica jalla) and three sub-species of

Black-winged Myna (Acridotheres melanopterus).

A number of internationally renowned zoos and local breeding centres contributed to the establishment of ex-situ populations for songbirds threatened by the trade. Currently, several facilities in Southeast Asia are running conservation breeding programmes for the ASTSG's priority species, including Greater Green Leafbird (Chloropsis sonnerati), Straw-headed Bulbul (Pycnonotus zeylanicus), Maratua Shama (Kittacincla (malabarica) barbouri), Barusan Shama (Kittacincla (malabarica) melanurus), Javan Pied Starling (Gracupica jalla), Wangi-Wangi White-eye (Zosterops sp. nov.), Javan Green Magpie (Cissa thalassina).

The ASTSG's trade and legislation team conducted multiple market surveys, including online, and analysed seizure data. Although much of the work focused on the trade in Indonesia, trade in other countries and international trade were also researched, including China, Malaysia, Thailand, Viet Nam and others. The findings supported legislative and law enforcement actions against those smuggling and selling songbirds without permits.

Demand reduction and consumer behaviour change studies were conducted in Indonesia, Singapore and Viet Nam, forming the basis of future behaviour change interventions. Demand reduction, education and community engagement initiatives were launched at different scales by organisations ranging from European zoos to local NGOs.

David Jeggo, Chair of the ASTSG and the report's co-author, believes that "This long-awaited report will be useful for academics and conservationists researching and tackling the unsustainable songbird trade in Southeast Asia, as well as for other stakeholders involved in the trade." The report also briefly outlines the direction and plans for the group in upcoming years.
The report is publicly available on the ASTSG's website: https://www.asiansongbirdtradesg.com/news For further inquiries, please email asiansongbirdtradesg@gmail.com

HANDFEEDING DAY ONE CHICKS

Click photo to follow link

Jordan Daniel

If anyone is looking for a formula for day 1 chicks that isn't coarse, and is smooth/silky in consistency, I highly recommend this formula. Made in Australia. As of late I had been using another brand that just wasn't doing well for baby Lories. Usually hit or miss. I ended up with a "star gazing" chick. The cause can be malnutrition (lack of vitamin D). I purchased organic banana baby food, switched back to Higgins high energy formula and purchased neocare. I mixed the three together (about half a tablespoon of each) for the last three days, and luckily the "star gazer" is now postured normally and doesn't throw itself back. Hand feeding formulas are NEVER a "complete" diet, and it can be frustrating when certain formulas effect certain species differently. Hopefully this information helps

I previously stated it was Vitamin D Deficiency from sources I found online. However it was pointed out to me that the cause of stargazing chicks is Vitamin B1 thiamine.
Jordan
Jordan Daniel
Lack Of *

https://birdpalproducts.com/search?type=product&options%5Bprefix%5D=last&q=neocare

Photo: Steve Duncan

I made this infographic to illustrate a phenomenon that many of you might find interesting in general because of how blue feather coloring works. There are no blue feather pigments so how do birds produce blue feathers? Blue color in feathers is the result of refraction of light like when a prism separates white light into the spectrum of colors. In very basic terms, the microscopic internal feather structure separates light like a prism and only allows the blue portion of the spectrum to escape and be seen. This is also why the angle of light can have a dramatic effect on how blue the same feather can look.

There is a question about the presence in the US of a mutation called Violet in Greencheek Conures that deepens and darkens their color, especially blue color. I am not making this post to claim that Violet does or does not exist in the US. I am only making this post because I see a lot of confusion caused by photos showing how deep the blue coloring of a single bird is thus claiming it has the Violet mutation. Judging the deepness of the blue coloring of a single bird in a photo is not accurate at all because lighting has a huge impact. A non-Violet bird can look Violet in the right lighting. A Greencheek (or any other parrot) with the Violet mutation will have deeper color in all light and all feathers when compared to a bird that does not have the Violet mutation. It doesn't just affect the long flight feathers for example. Photographing that difference is best done with 2 birds (a Violet and a non-Violet) next to each other in the same light.

Confounding the issue is that Greencheek Conures also have a mutation called Turquoise or Par-

blue which is a mutation of the Blue gene. The normal green color of a parrot is made by combining blue and yellow colors to get green. The Turquoise mutation reduces the amount of yellow pigment leaving the blue color more visible. The amount that the yellow pigment is reduced is variable so different Turquoise Greencheeks can look more or less blue when compared to each other.

So far, all photos I've seen of Greencheeks labeled as Violet in the US (there have been many by various people recently) have only been of Turquoise series birds which already vary tremendously in color. I have seen no photos of US birds that are Violet Green or Violet Opaline (Yellow-sided), for example. The way to prove you have a Violet mutation and not just a variation of Turquoise is to produce a Violet mutation bird without having it combined with other mutations that alter overall color. The inherent variability of Par-blue mutations, such as Turquoise especially, needs to be kept out of the test case so it isn't what's creating variability in how deep blue the bird appears. The proof should be producing a Violet Green or a Violet Opaline which will look different than a normal Green or Opaline. Crossing a Normal Green bird with a very blue Turquoise bird assumed to be Violet but that doesn't actually have the Violet mutation will result in normal Green offspring confirming that Violet is not present. Crossing a Normal Green bird with a Violet bird of any other mutation combination should result in some Violet Green offspring, thus proving the presence of the Violet mutation.

If you have to get a Turquoise bird in just the right lighting to see the coloring from a supposed Violet mutation, then perhaps it's actually the angle of light that is having the greatest influence on the color you are seeing instead of the Violet mutation. Or perhaps not, but a photo of a single Turquoise (blue series) bird is not the way to prove it carries the Violet gene. Buyer beware.

Normal (left) and variations of Turquoise Greencheeks

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EDITOR'S NOTE: Airfares are reportedly dropping just in time for fall travel. You've got to come to the 15th Annual Avicultural Society of America Educational Conference

Birds in Shoes

Jim Sorensen

My newest, iNhlekabafazi in Hiking Boots. This was a special request from Donna Rice. Last year she went with six women best friends/birders to Kruger National Park. For part of it, they did a wilderness hike and the Ranger called them the iNhlekabafazi (pronounced in schlock a baaa fazzi) which is a Zulu term meaning the raucous laughter of women (and their name for the green wood hoopoe because of their call). One of the things that kept cracking them up is one member kept dropping her lens cap so that was added. www.jimsorensen.com

Thank you, Jim Sorensen for allowing ASA to share your beautifully creative images!

Who's Your Daddy?

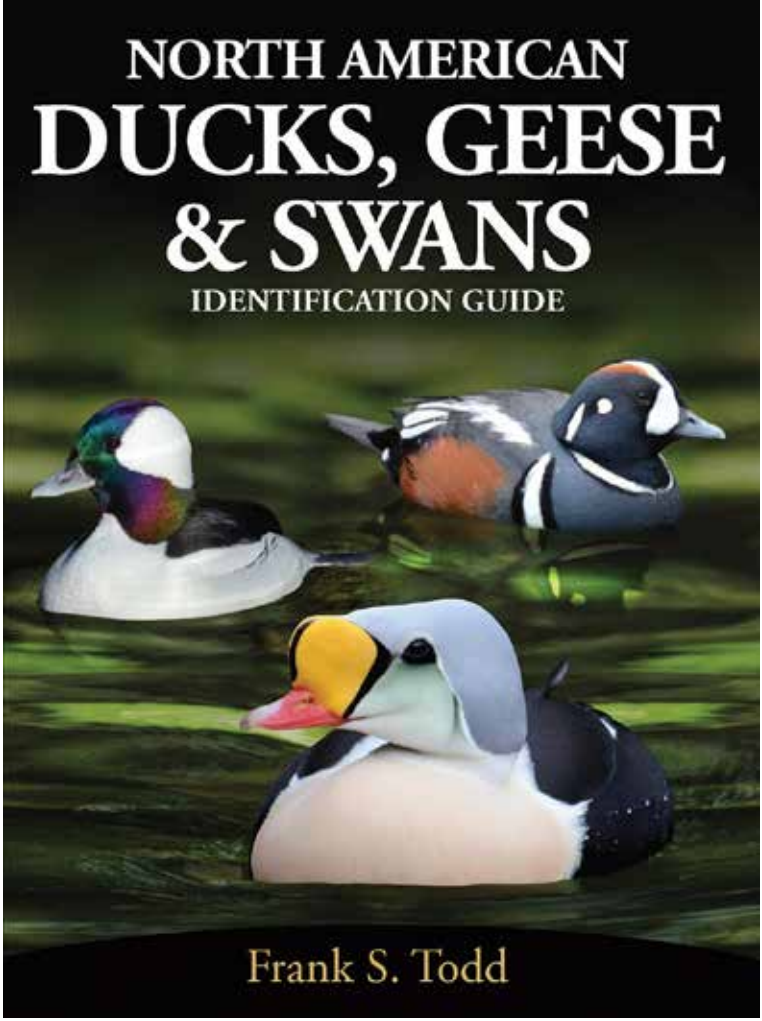
Stumped? See answer on page 44

Photo: Grover Wonderlin

HOMES for Animal Heroes

A National Animal Interest Alliance (NAIA) Initiative

http://www.homesforanimalheroes.org/ Homes for Animal Heroes is the first and largest nationwide network for rehoming research dogs that supports biomedical progress and all of the heroes who make it possible. It's time for transparency and time for us to share our love for animals and people…with the world. Thank you for supporting our vision of truth!



In honour of our friend, colleague, and author, Frank Todd, Hancock House is pleased to commit a percentage of all revenues of books sold through our website to the Frank Todd Memorial Foundation to continue to promote the work Frank spent much of his life striving towards wildlife conservation and education. You can purchase Ducks, Geese & Swans of North America: Identification Guide at: https://www.hancockhouse.com/collections/ducks-waterfowl/products/north-american-ducks-geese-swans

PLEASE DONATE NOW

Help us keep Frank S. Todd's memory alive by continuing the tradition he started with the first Avicultural Society of America Educational Conference. Frank developed the conference and, for many years, arranged for speakers from around the world to attend and make presentations. Your donation will allow ASA to continue the tradition and help with travel expenses for our conference speakers. http://asabirds.org/frank-s-todd-memorial-fund/

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Yellow-naped Amazon blue mutation (Amazona auropalliata)
Photo: Barbara Brady-Smith

Who's Your Daddy?

From page 39, Answer:
Yellow-naped Amazon blue mutation (Amazona auropalliata)

The Blue Mutation Yellow-Naped Amazon, (Amazona ochrocephala auropalliata), is one of most sought after parrots in the world. These Blue Amazons are more intelligent than most of their green relatives and their gorgeous blue color and larger size give them an impressive presence that cannot be captured in a photo.

SIZE & PERSONALITY
They are more intelligent and larger than most Yellow-Napes and that has been the opinion of all that have worked with them. Their talking ability and desire to engage in speech is incredible and they will talk whether you choose to formally teach them or not. This is also true of those that are kept as future breeders and therefore not kept tame or interacted with as family or personal pets.

COLOR
They are all blue where they would normally be green and they are white where they would normally be yellow or red. When two of these Blue Amazons are bred together, all of the offspring are always blue.

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SEE PAGE 39

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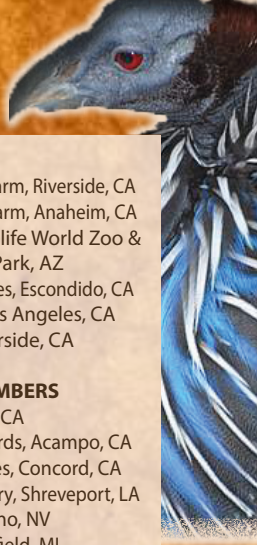

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