

## QUANTIFYING BETWEEN ANIMAL VARIATION FOR MALE SEMEN TRAITS AND FEMALE EGG TRAITS IN AN OSTRICH FLOCK USED TO DEVELOP AN ARTIFICIAL INSEMINATION PROTOCOL

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### SUMMARY

The development of a viable protocol for artificial insemination (AI) of ostriches may assist to overcome challenges to a structured breeding programme in this species. Data were obtained from up to 16 males trained to ejaculate in an artificial cloaca and 36 females producing eggs in the absence of males being used as a resource flock to develop an AI protocol. Repeatability estimates across years (termed as permanent environment or  $pe^2$ ) or within years (termed as temporary environment or  $te^2$ ) were estimated to assess whether current flock gains could be achieved by selecting particular animals. Male semen volume and libido were mostly affected by long-term  $pe^2$  effects at respectively 0.38 and 0.32, while sperm morphology and sperm concentration mostly depended on  $te^2$  effects, ranging from 0.09 for sperm concentration to 0.41 for the percentage normal spermatozoa. Permanent environmental effects were more important for semen motility traits, ranging from 0.03 for amplitude of the lateral head to 0.20 for straightness. However, most derived estimates did not differ from zero. Analysis of monthly female egg production and average egg weight records yielded estimates of 0.21 and 0.45 for  $pe^2$  and 0.11 and 0.15 for  $te^2$ , respectively. Selection for better adaptation to the proposed AI protocol may result in current flock gains for some male and female traits.

### INTRODUCTION

Genetic evaluation of ostriches is poorly developed when compared to other farmed livestock species (Cloete *et al.* 2008). Challenges associated with ostriches are a communal nesting system not conducive to the recording of parentage in colony mating flocks, confounded random effects and mate incompatibility when mated as pairs to record pedigrees, a very narrow male:female ratio and the absence of a formal recording and evaluation scheme (Bunter and Cloete 2004; Cloete *et al.* 2008). These challenges could all be alleviated by a workable protocol of artificial insemination (AI), which is studied as a viable alternative to natural mating (Malecki *et al.* 2008; Malecki and Rybnik-Trzaskowska 2011). Such technology also has the potential to reduce the male to female ratio and may add additionally to worker occupational health and safety by reducing the number of dangerous males, as well as bird welfare, by reducing incompatibility between animals paired off (Cloete and Malecki 2011). AI contributed markedly to livestock genetic improvement (Verma *et al.*, 2012). It is foreseen that the ostrich industry is likely to benefit from AI in the same way.

Since stress-free collection of semen in ostrich males has been developed (Rybnik *et al.* 2007), there have been a number of distinct advances towards making AI a reality in this species. It has, for instance, been demonstrated that semen can be collected from males trained to the dummy female method twice daily, without compromising ejaculate output and sperm quality (Bonato *et al.* 2011). Also, semen could be collected from trained males all year round, although some seasonal variation do occur (Bonato *et al.* 2014b). Stress-free artificial insemination of trained

ostrich females is possible by using the voluntary crouch principle (Malecki *et al.* 2008; Bonato *et al.* 2014c). Furthermore, inseminated females were demonstrated to produce fertile eggs (Bonato *et al.* 2014c).

However, individual variation is observed for all traits that are likely to be of importance and value in an AI program. The question remains whether between-animal variation can be exploited to select individuals well-adapted to AI. To date, all genetic parameters for ostriches have been derived from a single resource flock, employing a pair-breeding system (Cloete *et al.* 2008). It is unknown whether between-animal variance ratios estimated for pair-bred ostriches would also be applicable to usage in an AI program.

For this reason, in this study, the repeatability of sperm traits in males and egg traits in females was studied in a resource flock maintained to develop and refine a viable AI protocol in this species. Repeatability is the upper limit for heritability in cases where no animal permanent environmental effects are present.

## MATERIAL AND METHODS

**Location, animals and data collected.** The animal resource was maintained at the Oudtshoorn Research Farm of the Western Cape Department of Agriculture near Oudtshoorn in the Klein Karoo region of South Africa. Data were sourced from seven males trained to ejaculate in an artificial cloaca and used in the seasonal variation trial of Bonato *et al.* (2014b) for the assessment of ejaculate volume, sperm concentration, sperm morphology (the percentage of live, normal and dead spermatozoa) as well as male libido (4-point scale) over a period of 24 months. Sixteen males provided 1169 recorded ejaculates for use in this analysis. A total of 257 semen samples from 10 males collected from 2013 to 2015 were used to assess motility traits by using computer assisted sperm analysis (CASA) software (Microptic S.L. System Version 5.2, Barcelona, Spain). Traits considered were progressively motile and motile spermatozoa; curvilinear, straight line and average path velocity; linearity, straightness and wobble as well as beat cross frequency. These sperm motility traits are commonly related to fertilization success in other species (Suarez and Pacey 2006). Monthly egg production and egg weight (expressed as trait of the female) records were collected over 5 consecutive breeding seasons (from May to December, 2009-2013) in a female flock producing eggs in the absence of males (Bonato *et al.* 2014a). In total, 664 female-month records of 36 females were available for analysis.

**Statistical analyses.** Repeated records produced by the same male or female were accommodated in two ways by using ASReml software (Gilmour *et al.* 2006): firstly, the variance component of unique animals across years was considered as an indication of the animal permanent environment ( $pe^2$ ) and secondly, the correspondence of records of unique animals within production years were considered as an indication of animal temporary environment ( $te^2$ ). The former parameter can be considered to reflect long-term effects of specific animals on traits while  $te^2$  reflect short-term effects. No attempt was made to partition the  $pe^2$  variance component in genetic and permanent environmental effects, given the relatively small size of all databases (<1200 records) and the number of animals recorded (<40). Log likelihood tests were used to assess the significance of random effects added to the fixed effect analytical model. Fixed effects included were year (specific for the respective data sets), month or season and whether samples used for CASA were diluted or not. Fixed effects solutions are not relevant for this paper and therefore not reported.

## RESULTS AND DISCUSSION

There is little knowledge about the repeatability of sperm traits in ostrich males under repeat sampling. Between-male differences for sperm traits reported by Bonato *et al.* (2011; 2014b) suggest that such traits are likely to be repeatable. Estimates of  $pe^2$  and  $te^2$  for sperm traits and

libido are presented in Table 1. The results in Table 1 show that  $pe^2$  effects predominated in semen volume and libido scores. In contrast,  $te^2$  effects were more important for sperm concentration and morphology. Nonetheless, male specific semen traits were repeatable, suggesting that these records can be used to select males with a high semen yield and/or libido for usage in the current flock. No comparable work on ostriches, other ratites or avian species could be found.

**Table 1. Repeatability estimates ( $\pm$ s.e.) for semen volume, sperm concentration, sperm morphology traits and libido for the unique male across years ( $pe^2$ ) and the unique male within years ( $te^2$ ) effect, for males trained for the routine collection of semen using the dummy female method**

Trait	$pe^2$	$te^2$
Semen volume (mm)	0.38 $\pm$ 0.12	0.13 $\pm$ 0.06
Sperm concentration ( $\times 10^9$ /ml)	–	0.09 $\pm$ 0.03
Normal sperm (%)	–	0.41 $\pm$ 0.08
Abnormal sperm (%)	–	0.31 $\pm$ 0.06
Dead sperm (%)	–	0.38 $\pm$ 0.07
Libido score (n)	0.32 $\pm$ 0.10	–

The between-male variance of male sperm motility traits mostly partitioned towards  $pe^2$  (Table 2). Derived estimates were, however, relatively low ( $<0.20$ ), and only reached the level of double the corresponding s.e. for straightness. Although mostly not significant ( $P<0.05$ ) when related to the corresponding s.e., the inclusion of the additional random term improved the log likelihood ratio for all traits except the amplitude of lateral head, and wobble. The  $pe^2$  variance ratios for all traits associated with velocity were below 0.10. The only trait primarily affected by  $te^2$  was linearity. We did not find any literature on similar traits in ostriches or other ratites.

**Table 2. Repeatability estimates ( $\pm$ s.e.) for male sperm motility traits according to CASA for the unique male across years ( $pe^2$ ) and unique male within years ( $te^2$ ) effect, for males trained for the routine collection of semen using the dummy female method**

Trait	$pe^2$	$te^2$
Progressive motile spermatozoa (%)	0.13 $\pm$ 0.08	–
Motile spermatozoa (%)	0.19 $\pm$ 0.10	–
Curvilinear velocity ( $\mu$ m/s)	0.08 $\pm$ 0.07	–
Straight line velocity ( $\mu$ m/s)	0.09 $\pm$ 0.07	–
Average path velocity ( $\mu$ m/s)	0.07 $\pm$ 0.06	–
Amplitude of lateral head ( $\mu$ m)	0.03 $\pm$ 0.05	–
Linearity (%)	–	0.12 $\pm$ 0.09
Straightness (%)	0.20 $\pm$ 0.10	–
Wobble (%)	0.05 $\pm$ 0.05	–
Beat cross frequency (Hz)	0.10 $\pm$ 0.07	–

Between-female variance components for egg production and average egg weight partitioned mostly to  $pe^2$  and to a lesser extent to  $te^2$  (Table 3). The estimate of  $pe^2$  were approximately double that of  $te^2$  for egg production and about three times as high for average egg weight. The derived parameters were close to a  $pe^2$  estimate of 0.17 (the sum of  $h^2$ ,  $pe^2$  and the service sire variance ratio) and a  $te^2$  estimate of 0.15 derived by Fair *et al.* (2011) for monthly egg production records of pair-mated ostriches. Corresponding estimates by Fair *et al.* (2011) for egg weight amounted to 0.57 for  $pe^2$  and 0.13 for  $te^2$ , which also corresponded fairly well with the present estimates. From these results it seems as if parameters derived for a flock of females producing eggs in the absence of males are quite similar to those derived for pair-mated females.

**Table 3. Repeatability estimates ( $\pm$ s.e.) for monthly female egg production for the unique female across years ( $pe^2$ ) and unique female within years ( $te^2$ ) effects, for females maintained without males and producing eggs in the absence of males**

Trait	$pe^2$	$te^2$
Egg production (n)	0.21 $\pm$ 0.07	0.11 $\pm$ 0.04
Average egg weight (g)	0.47 $\pm$ 0.08	0.15 $\pm$ 0.04

## CONCLUSION

This study is the first report of between-animal variation available for exploitation in an AI program for ostriches, aimed at overcoming typical production challenges, while also promoting genetic progress as in other species. Key male traits like semen volume and libido were repeatable across years, while sperm morphology traits were more affected by short-term animal affects. Between-animal effects for sperm motility traits were smaller and mostly not significant. Further work on these traits are needed to ascertain their role in fertilizing eggs produced by females subjected to AI. Parameters for female ostriches in the AI program were consistent with estimates from a pair-breeding flock. This is good news for the development of a viable AI program in this species, as current-flock gains are expected to selection for egg traits in female ostriches producing in the absence of males.

## ACKNOWLEDGEMENTS

This research was partly funded by the Western Cape Agricultural Research Trust, the South African Ostrich Business Chamber and the THRIP initiative of the National Research Foundation. We are also indebted to Miss Naomi Serfontein for assistance with the husbandry of the flock.

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