INTRODUCTION

All genetic information from each bird is derived from each parent, except the mitochondrial DNA (mtDNA), which is passed via the maternal line only. Since mitochondria are the ‘power stations’ in each cell and hence essential to the performance of those cells (Kappler et al., 2019), one could imagine that genetic variation mtDNA could potentially be a factor in differences in race performance. This hypothesis is accepted by many in the pigeon racing world without being tested. Many fanciers believe that the maternal line of racing pigeon families is the genetic key to race performance of their offspring in particular on the longer distances; thus, leading to the preference to add hens from the maternal line to the breeding loft to pass on the specific mtDNA (Mattacchione & Hechanova, 2010). However, this hypothesis has never been proven scientifically in a prospective study.

The objective of this prospective study is to explore the race performance of those birds carrying the mtDNA from the famous hen Queen L (Jelle Roziers) compared to the race performance of birds from the same family not carrying this mtDNA. Queen L won 1st National La Souteraine against 16,297 birds and her offspring includes many champion racers in many generations down.

In 2019 in one selected breeding loft, all 17 breeding pairs were related to Queen L. In these 17 pairs the relationship with Queen L was either via the paternal line (not passing on the Queen L mtDNA) or via the maternal line (passing on the mtDNA). This closely bred family of birds is hence ideal to address this question whether presence of Queen L mtDNA makes a difference in race performance, in particular on the longer distances. A control group was identified on the second most frequently present mtDNA i.e. from National Treasure (winner of 2nd place National Chateauroux against 17,109 birds) a famous racing and breeding hen by Bart Geerinckx (Belgium). Pedigree review indicated that no other maternal line had a substantial presence in the breeding loft.

This is the first prospective study to investigate the impact of mitochondrial DNA (mtDNA) on race performance in a closely bred family of racing pigeons in one breeding loft where there is a high percentage of male and female birds related to one foundation hen, Queen L by Jelle Roziers. This study shows that in this family of birds, which are all related to Queen L, there is no advantage of having mtDNA with regard to race performance on the longer distances. The results of this study reject the hypothesis that mtDNA genes are related to long-distance performance. Actually, this analysis indicates that those birds in this family with no mtDNA from Queen L may have better race performance on the shorter distances than those with mtDNA. This suggests that there are other genetic factors which may be related to race performance in this family of racing pigeons.

KEYWORDS
mitochondrial DNA, pigeon racing, race performance
2 | MATERIAL AND METHODS

In 2019, 17 breeding pair produced 124 young birds. Of these, 107 were entered in so-called one of 14 One Loft Races (OLRs) and were raced at an age of less than 12 months. These 107 birds were included in this analysis. Each bird was given a status of lost before races, lost during races or race series completed. Performance measurement (marker of performance) is based on race coefficient (RC) calculated as place won divided by number of birds in the race. Hence a lower number indicates a better performance. This is a continuous measurement of performance rather than an artificial ranking or point system. Results are reported over all races combined and by race distance. Medians are reported for each group as medians are a better reflection of group performance than means, and less susceptible to individual outlier values. Races were grouped in the distance categories: short distance (70–160 miles); middle distance (161–260 miles), long distance (261 miles and more).

Determination of mtDNA status is based on the maternal lineage in the pedigree. The hypothesis is that birds with Queen L mtDNA present have a better performance on longer races than those without.

For the primary analysis of race coefficients for the effect of mtDNA, the method of Kruskal and Wallis (1952) was applied. Box plots were generated to display outcomes among the various factors of interest. P-values were unadjusted for multiple testing. Statistical analyses were carried out with a control group with mtDNA from National Treasure and a group with neither mtDNA from Queen L nor from National Treasure.

3 | RESULTS

Of the 17 breeding pairs, 16 breeding cock birds were related to Queen L (but not passing on mtDNA) and of the 17 hens, 11 were related to Queen L, but only 5 were passing on her mtDNA. For the control group there were 3 breeding hens passing on mtDNA for National Treasure.

The 17 pairs produced 124 birds of which 122 birds had DNA samples collected; 107 birds were sent to OLRs and raced at age <1 year old, 2 were sent to local races, 10 were sent to a yearling race (and raced at >1 year of age), 3 were not sent to any races. Of the 107 birds 23 carry the mtDNA, 84 birds did not. The data set consisted of 124 Pigeons with 636 total observations. Of the 636, 73 had data from which a race coefficient could be calculated (585 observations). Of the 73, 71 had mtDNA data (558 observations). Data analysis is based on the 558 observations from the 71 pigeons.

Demographics of 71 birds included in the analysis are presented in Table 1.

Table 2 contains the summary of race coefficients overall and the factors of interest. Race coefficients were non-normally distributed. Summaries of race coefficients include median, minimum and maximum. Comparisons of race coefficients among levels of any factor were made using a Kruskal–Wallis Test. Box plots were used to display distributions.

For the primary analysis of race coefficients, differences were seen for mtDNA alone showing that those without Queen L mtDNA had a lower RC (=better performance) than those with mtDNA (Figure 1). This difference was significant (p = 0.004). Exploring these genes in a multivariate model with other demographic and genetic factors, the difference based on mtDNA remained significant.

Looking further into mtDNA effect at the different race distances (Figure 2), the effect of mtDNA was only observed in the shortest races (Kruskal–Wallis p = 0.034). There were no differences between birds with or without mtDNA in long-distance races. This observation may be a function of due to a smaller number of observations for the long and medium race lengths.

In this analysis, no difference was found between RCs from birds with mtDNA of National Treasure and birds without (Kruskal–Wallis Test p = 0.57) indicating that the RC results for control group for Queen L mtDNA analyses were consistent and not driven by the subgroup of birds with mtDNA of National Treasure.

In the three-group analysis by distance, between birds with mtDNA from Queen L, National Treasure or Neither mtDNA, no differences were found for long distance or middle distance (Figure 3). The only difference found was the birds with Queen L mtDNA had higher RCs in short-distance races (Table 3). For the long-distance races, the median RCs were 0.280 (Queen L mtDNA), 0.253 (National Treasure mtDNA) and 0.314 (Neither mtDNA); these medians were not significantly different (Kruskal–Wallis test p = 0.97).

4 | DISCUSSION

Results of this study are applicable to the family of birds studied. It is not known if these observations will be applicable to the wider pigeon populations. The study of mtDNA in this close bred family of birds is

| TABLE 1 | Characteristics among birds with an mtDNA assessment and racing data |
|----------|---------------------------------------------------------------|
|          | n = 71 | Queen L mtDNA | No mtDNA       |
|          |         | (n = 17)       | (=54)          |
| Hen : Cock birda | 32 (39) | 9 (8)         | 23 (31)        |
| Blue barb | 14 (20%) | 4 (24%)       | 10 (18%)       |
| Blue checkb | 54 (76%) | 10 (59%)     | 44 (81%)       |
| White    | 3 (3%)  | 3 (18%)       | 0              |
| Completed race series | 39 (55%) | 5 (29%) | 34 (63%) |
| Lost during race series | 32 (45%) | 12 (71%) | 20 (37%) |
| mtDNA National Treasure | 13 (18%) | 0 (0%) | 13 (24%) |

| a Gender based on genetic testing. |
| b Base colour, not including white flights or splash colour. |
appropriate, as other genetic factors should be evenly distributed and thus this population would be more homogenous than the general pigeon population. However, the relative small sample size coming from one loft only, may limit drawing conclusions in particular in the subcategories. For some context, we reviewed outside this study, the pedigrees of the 8 best performance pigeons in 2020 at Jelle Roziers loft in Belgium: Of these 8 birds, 4 have mtDNA from Queen L and 4 do not (Jelle Roziers, personal communication, October 2020). At a minimum, this observation is not contradicting the finding of our study.

Determination of mtDNA status is based on the maternal lineage in the pedigree. No molecular analyses to determine mtDNA profile or parenthood were conducted. On the one hand, this may be a weakness in determining the mtDNA status as paternal parenthood is not guaranteed in a loft setting versus individual breeding pens. On the other hand, since the mtDNA is maternal and there is no doubt of the origin of the eggs laid: all eggs laid were accounted for (no eggs were found to be laid in any other nest). Hence, establishing mtDNA status by pedigree was an acceptable approach.

Race performance can be measured in many ways, we chose a continuous measurement of Race Coefficient (position won divided by number of birds in the race), over any point or classification measurement, this to be most objective. Repeating this analysis using other measurements of race performance was not conducted. Birds were entered in one of 14 OLRs and were raced at an age of younger than 12 months. Diversification over different OLRs on one hand ensures that one OLR does not impact the study results, therefore we did not explore any potential impact of other factors known to influence race performance. Birds were entered in the races without regard to mtDNA status. The actual race performance in 2019 for the birds studied showed good distribution ranging across the whole spectrum of results, including several top positions (64 top 10% positions in US OLRs), supporting the validity of the database used for race performance.

This study rejects the hypothesis that Queen L mtDNA is related to better performance on the long distance. Actually this observation suggests that not having Queen L mtDNA may result in better performance in the short-distance races. The groups seem to be balanced for demographic factors as well as distribution over the difference OLRs. There seems to be an imbalance with more Queen L mtDNA birds lost during the race series. It is not known if, and if so, how much this has an impact on this study. All birds in the races were related to Queen L. Most of the breeders (27/34 = 79%) in this family are related to Queen L, and the coupling of birds was such that each pair had at least one bird related to Queen L. This results in the unique situation that the entire study population of birds was 100% related to Queen L. We can speculate that there may be another genetic factor(s) that determines race performance. Further study will be needed to explore such genotype–race performance relationship.

Additional analyses were conducted comparing the control group of birds with mtDNA of National Treasure and birds with Neither Queen L nor National Treasure mtDNA. These did not show any inconsistency that the results were driven by any particular subgroup. Actually for the long-distance races there was no difference observed between RCs of these three groups, further supporting the conclusions of this study.

In conclusion, this study rejects the myth of the relationship of mitochondrial DNA and long-distance race performance. These results show that within the same closely bred family of birds, those birds without mtDNA of Queen L have a better race performance in particular on the shorter races than those birds with Queen L mtDNA. The genetic factors determining race performance within the Queen L family remain to be determined, but mtDNA is not related to differences in long-distance race performance.

**ETHICS STATEMENT**

Study was conducted by individual scientists without institutional relationship. Ethics review was not available. Study was conducted following high standard and generally accepted practice to breed and race racing pigeons.
CONFLICTS OF INTEREST
None.

AUTHOR CONTRIBUTION
Geert Kolvenbag: Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Writing-original draft. Mark Scott: Formal analysis; Visualization; Writing-review & editing.

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Data available on request from the authors.

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FIGURE 3 Boxplot of RC on long-distance races by mtDNA for Queen L, National Treasure or neither

| TABLE 3 Summary of RCs per distance for birds with mtDNA from Queen L, National Treasure or neither |
|---|
| Distance | mtDNA | # Race Records | RC | p-value Kruskal-Wallace |
| Long | National Treasure | 12 | 0.253 | ns p = 0.97 |
| Long | Neither | 32 | 0.314 | |
| Long | Queen L | 10 | 0.280 | |
| Middle | National Treasure | 19 | 0.159 | ns p = 0.11 |
| Middle | Neither | 77 | 0.349 | |
| Middle | Queen L | 16 | 0.331 | |
| Short | National Treasure | 84 | 0.264 | p = 0.0075 |
| Short | Neither | 227 | 0.270 | |
| Short | Queen L | 81 | 0.474 | |

Abbreviation: ns, not significant.

a For short-distance races: Queen L RCs both significantly different from Neither (p = 0.0087) and National Treasure (p = 0.0236). No difference between National Treasure and neither.

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