

Iron overload in captive Amargosa voles (*Microtus californicus scirpensis*)

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Background

- The endangered Amargosa voles (*Microtus californicus scirpensis*) are native to the marshes of the Mojave Desert where they live 3-4 months on average and occasionally greater than 12 months. They subsist on a diet of bulrush.¹
- As part of a species recovery effort², a captive breeding colony of 20 founders was established at UC Davis. Colony health surveillance and disease diagnostics is provided by the Comparative Pathology Laboratory (CPL).
- Voies in the colony are provided a diet of commercial rabbit and rodent chow and were documented living up to 35 months.³
- Within the past year, two voles were diagnosed with iron storage disease (hemochromatosis) based on elevated hepatic iron associated with hepatocellular damage.
- Iron storage disease may generally be attributed to excessive dietary iron or a hereditary malfunction in iron absorption and storage pathways.⁴

Objectives

Objective 1: Define and confirm the presence of iron storage disease in the captive Amargosa vole breeding colony.

Objective 2: Identify risk factors for iron storage disease in captive Amargosa voles.

Hypothesis: Iron overload in this Amargosa vole colony is associated with time-in-captivity in the context of a normoferric diet.

Methods

- Mass spectroscopy was used to measure hepatic iron content of the two affected Amargosa voles, an affected Prairie vole (*Microtus ochrogaster*), three age and sex matched unaffected voles which served as controls, and three age and sex matched B6 mice which served as additional controls.
- From 72 voles in the CPL archives, 44 were randomly selected from five time-in-captivity groups: (A) 0-6 months, (B) 7-12 months, (C) 13-18 months, (D) 19-24 months, and (E) 25+ months. Group A included six voles, groups B and C contained ten voles, and groups D and E consisted of nine voles. Twenty-two voles were female and twenty-two were male.
- Formalin-fixed liver tissue was sectioned and stained with Perl's iron. Each slide was evaluated for iron content at three of the most affected sites using CellSens software. A color threshold was set to measure only the area of dark blue within each field. The software produced the percent region of interest (ROI %) for each image and the average ROI % was calculated for each individual.
- Historic lineage data was qualitatively evaluated for relatedness between the affected voles.

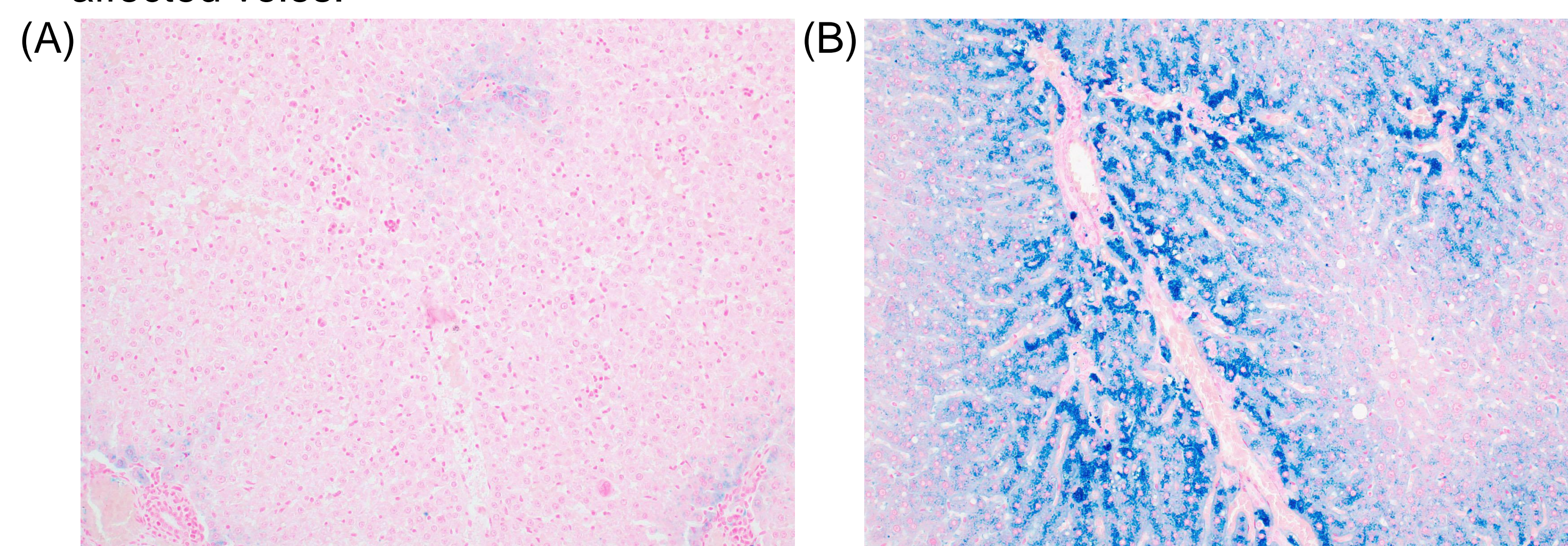


Figure 1. Perl's iron-stained Amargosa vole liver tissue at 20x magnification. A, ROI 0.72%. B, ROI 24.95%.

Results

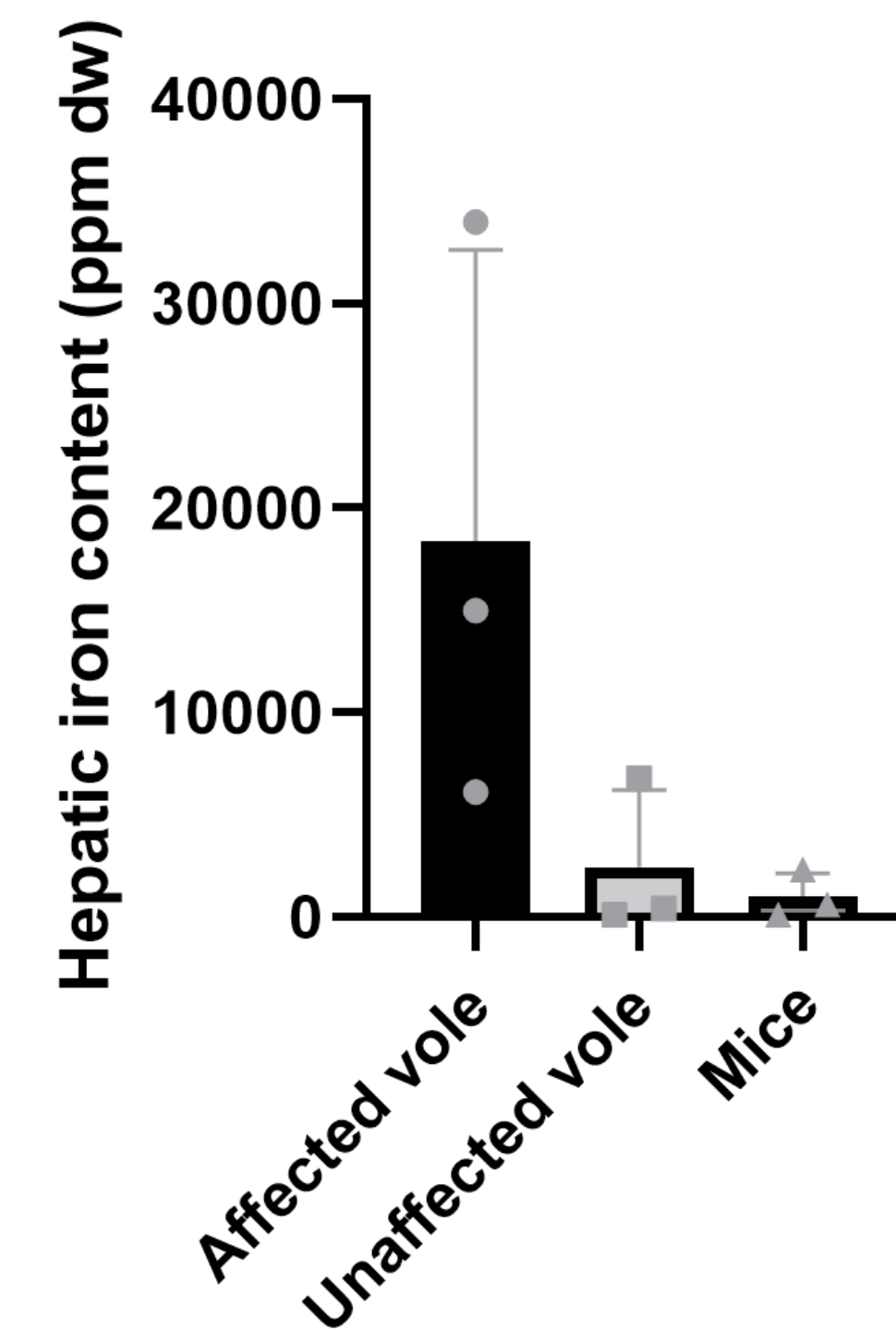


Figure 2. Average hepatic iron content of three age and sex-matched affected *Microtus* voles, three unaffected *Microtus* voles, and three B6 mice as measured by mass spectroscopy. Analysis of variance reveals the differences in hepatic iron content between the three groups are trending towards significance ($P=0.0851$).

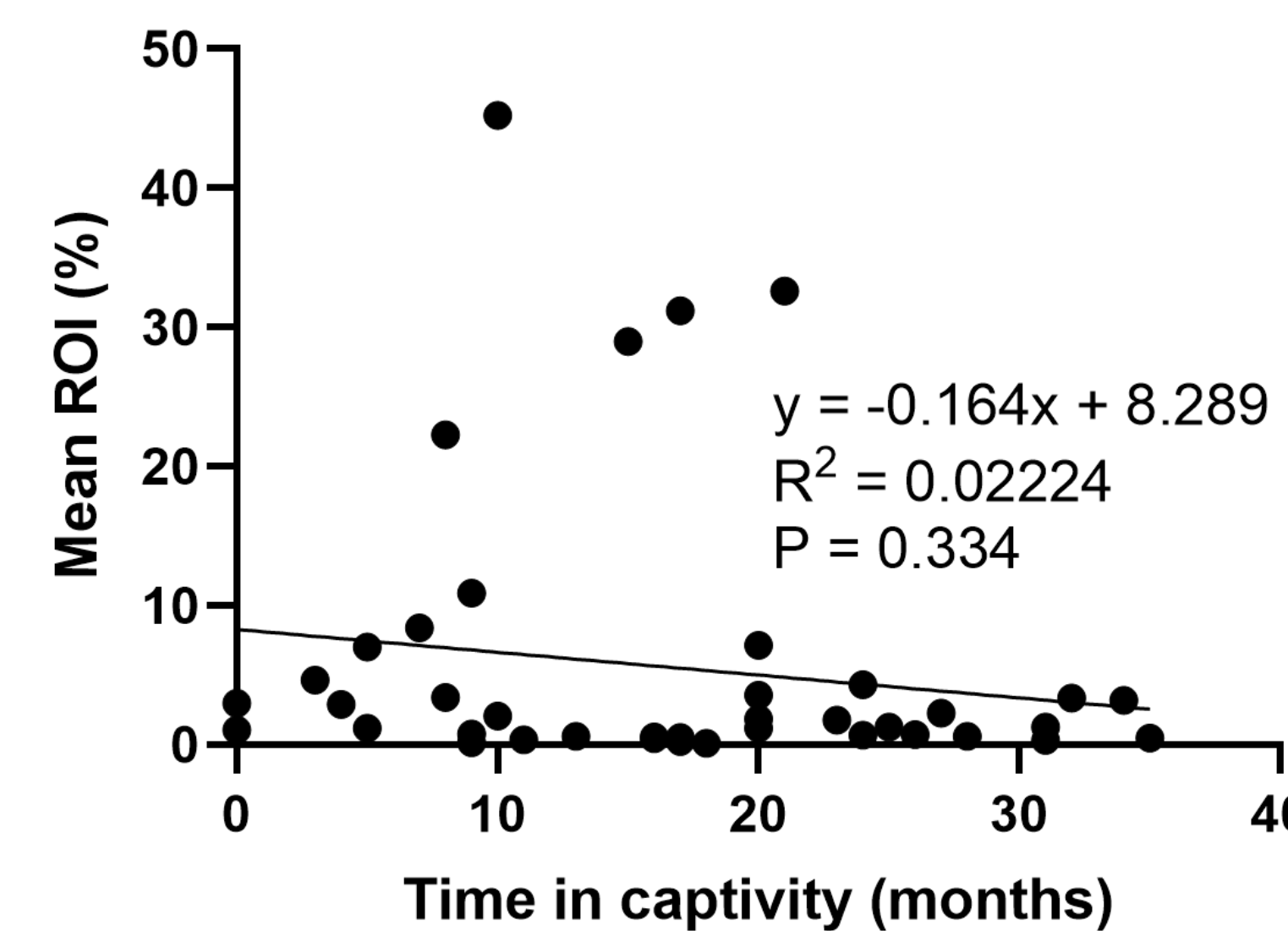


Figure 3. Hepatic iron content of Amargosa voles as measured by image analysis of Perl's iron stained liver tissue over time in captivity. Analysis of variance reveals no significant difference in hepatic iron content between the five time-in-captivity groups ($P=0.5434$). Linear regression indicates no significant association between time-in-captivity and hepatic iron content ($P=0.33$). The five outliers were identified as affected individuals; the mean ROI % of the affected group is significantly different than the mean of the unaffected group ($P<0.0001$).

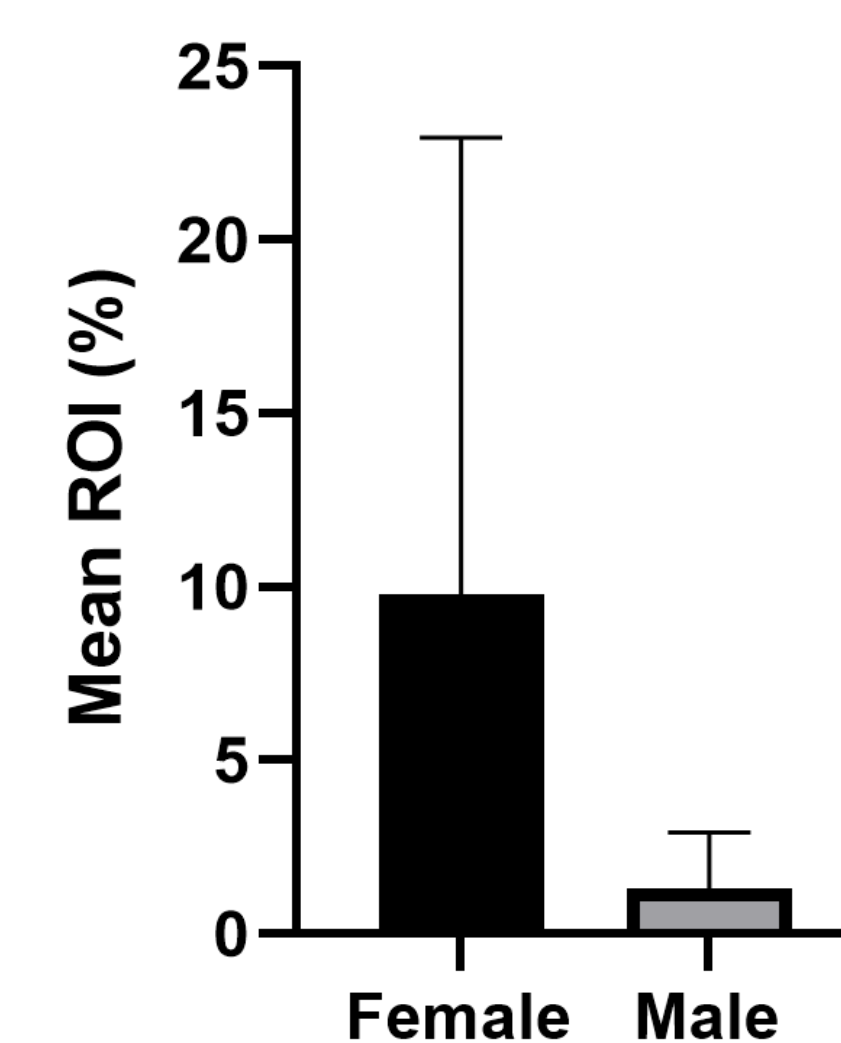


Figure 4. Mean hepatic iron content and standard deviation of male and female Amargosa voles as measured by image analysis of Perl's iron stained liver tissue. T-test reveals a significant difference in average hepatic iron content between sexes ($P=0.0046$).

Results (continued)

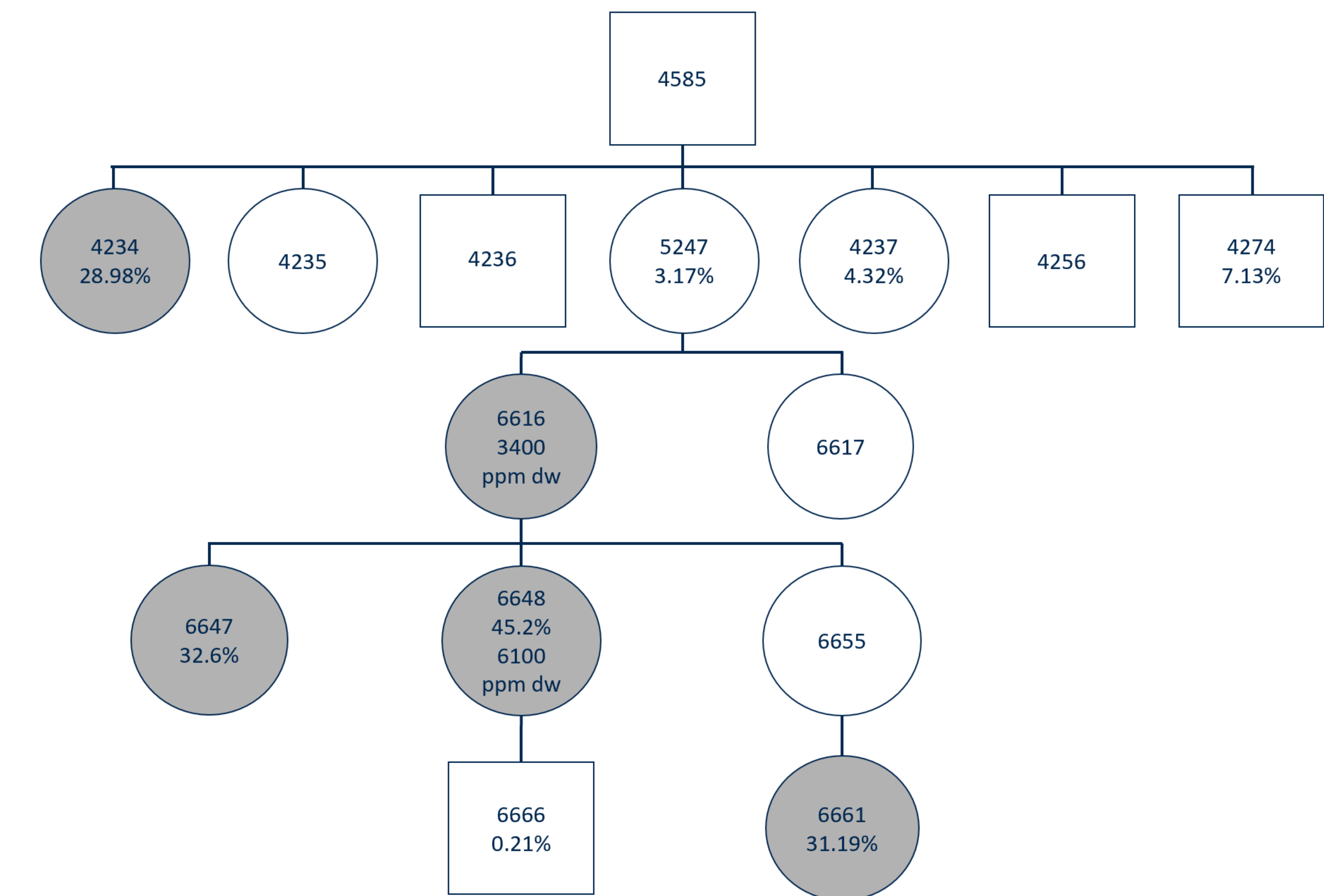


Figure 5. Pedigree of affected Amargosa voles. Affected individuals (gray background) are all related to a single founder. Hepatic iron content measured either by mass spectroscopy or analysis of Perl's iron stained liver tissue is listed for affected individuals.

Conclusions

- Iron storage disease is present in one related lineage within the captive breeding colony of Amargosa voles.
- Elevated hepatic iron content is not associated with time-in-captivity.
- Elevated hepatic iron content is associated with sex, with females being more affected than males.
- The cause of iron storage disease in the Amargosa voles is likely hereditary and less dependent on the diet.

Acknowledgments

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References

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